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THE CHARACTERIZATION OF WASTE ACTIVATED SLUDGE PRETREATED BY ULTRASOUND

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ABSTRACT

Excess activated sludge pretreatment in the wastewater treatment process aims at enhanced performance in sludge handling and in the stabilization of excess sludge. Excess sludge pretreatment may increase subsequent aerobic or anaerobic digestion, which is used for sludge stabilization before disposal. This study concentrated on the use of ultrasound as a pretreatment method and the aim was to characterize activated sludge before and after ultrasonic treatment of different ultrasonication duration times. The most desired effects are increased biodegradability, better dewaterability, and reduced sludge quantities.

The use of ultrasound in sludge treatment is based on cavitation phenomenon, which induces the formation of cavitation bubbles. Imploding bubbles produce shear forces, and high local temperatures and pressures that break up flocs and cell structures. The use of ultrasound requires choosing operational parameters, including for example ultrasonic power and duration, as well as sample volume and sludge total solids content. In this study, the main variable was ultrasonication time. Sludge characterization techniques included temperature, particle size distribution, TTF, SVI, solids content, COD, biodegradability and carbohydrate content.

Results showed that ultrasonic treatment increased sludge temperature, COD in supernatant and in filtrate, and carbohydrate content. The SVIs were also enhanced. Particle size distribution shifted towards smaller particles, causing the deterioration of filterability and supernatant turbidity during ultrasonication. Re-flocculation during ultrasonic treatment was observed. Ultrasonic treatment also caused partial inactivation of sludge during ultrasonication and the heating of sludge. Ultrasonic treatment had no effect on relative shares of total, volatile, and suspended solids.

Ultrasonication is a good method for releasing biodegradable material from solid phase to soluble form. The amount of SCOD proved to be a good way to analyze sludge disintegration and overall it can be used in the evaluation of biodegradable matter in sludge. In future, full scale ultrasonic applications should be studied in detail to establish whether operational conditions can be optimized to achieve a cost-effective treatment of excess sludge and enhanced sludge stabilization process.

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TIIVISTELMÄ

Jätevedenpuhdistuksessa poistettavan aktiivilietetteen esikäsittelyn tavoitteena on pyrkiä tehostamaan lietteen jälkikäsittelyä sekä lietteen stabilointiprosessin suorituskykyä. Ylijäämälietteen esikäsittely voi lisätä lietteen biohajoavuutta mädätys- ja kompostointiprosessissa ennen lietteen loppusijoittamista. Tässä tutkimuksessa keskityttiin tutkimaan ultraäänen käyttöä esikäsittelymenetelmänä. Tavoitteena oli karakterisoida aktiivilietenäytteitä ultraäänikäsittelyä ennen sekä sen jälkeen. Keskeiseksi muuttujaksi valittiin lietteen ultraäänikäsittelyaika. Tavoitteena oli lietteen parempi biohajoavuus, parempi vedenpoisto, sekä vähentynyt lietteen määrä.

Ultraäänen käyttö lietteen käsittelyssä perustuu kavitaatioilmiöön, joka muodostaa lietteeseen kavitaatiokuplia. Kuplien luhistuminen saa aikaan mekaanisia leikkausvoimia jolloin suuret paikalliset lämpötilat sekä paineet luhistuvien kuplien lähellä hajottavat lieteflokkeja sekä solujen rakenteita. Ultraäänen käyttöä varten valitaan toimintaparametrit, tärkeimpinä ultraäänen teho ja kesto, sekä ultraäänellä käsiteltävän näytteen tilavuus ja lietteen kuiva-ainepitoisuus. Lietteen karakterisointitekniikoina tässä työssä käytettiin lämpötilaa, hiukkaskokojakaumaa, suodatusaikaa, lietteen laskeutuvuutta, kiintoainepitoisuuksia, kemiallista hapenkulutusta, biohajoavuutta sekä hiilihydraattipitoisuutta.

Tässä työssä todettiin, että ultraäänikäsittely nosti lietteen lämpötilaa, lisäsi kemiallisen hapenkulutuksen määrää niin laskeutetun lietteen kirkasteessa kuin suodatetussa lietteessä, sekä lisäsi hiilihydraatin määrää kirkasteessa. Myös lietteen laskeutuvuus parani. Ultraäänikäsittely pienensi hiukkaskokoa, joka puolestaan heikensi suodattuvuutta sekä lisäsi kirkasteen sameutta. Tutkittaessa partikkelikokojakaumaa havaittiin myös lietteen uudelleensaostumista. Liete inaktivoitui osittain ultraäänikäsittelyn ja siitä seuranneen lietteen lämpiämisen takia. Ultraäänikäsittely ei vaikuttanut lietteen kuiva-aine- ja kiintoainepitoisuuksiin sekä orgaanisen kiintoaineen suhteelliseen määrään.

Ultraäänikäsittely vapauttaa biologisesti hajoavaa materaalia kiinteästä faasista liukoiseen muotoon ja liuenneen kemiallisen hapenkulutuksen määrän avulla voidaan analysoida lietteen hajoamista ja sitä voidaan käyttää biohajoavan aineen määrän arvioimiseen. Tulevaisuudessa täyden mittakaavan ultraäänisovelluksia tulisi tutkia yksityiskohtaisemmin, jotta voidaan arvioida voidaanko ultraäänikäsittelyn toimintaolosuhteiden optimoinnilla saavuttaa kustannustehokas lietteen käsittely ja tehostaa lietteen stabilisointia.

Preface

This study was executed in two parts, which consist of experimental and literary parts. All of the experiments were performed at the Universidad Rey Juan Carlos in Madrid, Spain. Literary work was finished at the Tampere University of Technology in Tampere, Finland.

First and foremost, I wish to express ample gratitude to my supervisor Professor Jukka Rintala for his guidance and support. I would also like to give special thanks to Professor Yolanda Segura at the University in Madrid for giving me a chance to work with her, and giving me possibilities and guidance during the experimental work. The help of laboratory staff at the Universidad Rey Juan Carlos is much appreciated and an occasional language barrier was defeated in the good spirit of co-operation. I am also thankful for Professor Tuula Tuhkanen at Tampere University of Technology for being a good help in the initial stages of the literary work.

Finally, I want to give warmest thanks to all my family and friends, for their support and for pushing me forward and giving me reinforcement during this process. Most of all, I want to thank my lovely daughter Sara for inspiration.

Tampere, April 2016

Suvi Ahtola

CONTENTS

ABBREVIATIONS

BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CST	Capillary Suction Time
DS	Dry Solids
EPS	Extracellular Polymeric Substances
OUR	Oxygen Uptake Rate
SCOD	Soluble Chemical Oxygen Demand
COD_f	Chemical Oxygen Demand in Filtrate
pp	percentage point
SS	Suspended Solids
SVI	Sludge Volume Index
TCOD	Total Chemical Oxygen Demand
TS	Total Solids
TTF	Time-To-Filter
VS	Volatile Solids

1 Introduction

Activated sludge produced in the wastewater treatment process has complex physical and chemical characteristics. Sludge consists mainly of water that is partially bound into sludge flocs. Sludge flocs consist of extracellular polymeric substances (EPS), micro-organisms, organic pollutants, inorganic particles and multivalent cations. EPS originate from microbial activity and they keep together microbial aggregates. EPS consist of proteins, polysaccharides, lipids, nucleic acids and biopolymers. All wastewater treatment plants are different and so is the sludge. Activated sludge characteristics depend on the composition of wastewater and the plant operating conditions. Both of these affect the size, density, micro-structure and surface properties of sludge. (Urbain et al. 1993; Chu et al. 2001; Jin et al. 2004; Subramanian et al. 2010.)

The removal of water from excess sludge and its subsequent aerobic or anaerobic treatments are the most costly operations in wastewater treatment and may sometimes be difficult to carry out. Excess sludge from wastewater treatment process is treated by aerobic or anaerobic digestion before utilization or final disposal. The purpose of the digestion is to stabilize sludge and reduce its volume. Micro-organisms consume all biodegradable material and the output is stabilized solids and carbon dioxide from the aerobic digestion or stabilized solids and biogas from the anaerobic digestion. The speed and the extent of both aerobic and anaerobic digestion depend on the hydrolysis of cells and EPS. Pretreatment of excess sludge can be used to enhance and increase the digestion performance. (Chu et al. 2001; Jin et al. 2004; Mao et al. 2004; Gröönroos et al. 2005; Zhang et al. 2007; Yu et al. 2008.) The present study concentrates on the use of ultrasound as a pretreatment method and on the characterization of sludge after ultrasonic treatment.

The characteristics of activated sludge have been studied intensively as well as the characterization of activated sludge after ultrasonic treatment. Most studies assessed the effects of the ultrasonic treatment on the anaerobic digestibility of excess sludge. Some studies also evaluated the effects of ultrasonic treatment on sludge dewatering qualities. The most desired effects of ultrasound on activated sludge would be enhanced biodegradability, reduced activated sludge quantities, better dewaterability, the release of chemical oxygen demand (COD), and the destruction of filamentous micro-organisms. (Chu et al. 2001; Mason & Pétrier 2004; Parsons 2004; Zhang et al. 2007; Show et al. 2009.)

When ultrasound is used for sludge, treatment parameters such as the energy, frequency and the duration of the applied ultrasound need to be decided. (Chu et al. 2001; Gröönroos et al. 2005; Show et al. 2007; Pilli et al. 2011.) The propagation of ultrasound depends on sludge total solids (TS) content. Too high TS content disables the propagation of ultrasound and the effect of ultrasound diminishes. The most important factors affecting the effect of ultrasonication are sludge TS content, sludge viscosity and temperature, sludge mixing or flow, ultrasound frequency and power, and also the size of the reactor and the transducer type. (Gröönroos et al. 2005; He et al. 2011.) Ultrasonic treatment of activated sludge affects sludge temperature, particle size distribution, filterability, settling properties, COD and biodegradability. (Chu et al. 2001; Gröönroos et al. 2005; Dewil et al. 2006; Zhang et al. 2007; Feng et al. 2009b.)

The objective of this study was to evaluate the effect of ultrasonic treatment on activated sludge characteristics. The characterization of activated sludge was carried out before and after ultrasonic treatment. Characterization techniques included temperature and pH, TS, volatile solids (VS) and suspended solids (SS), particle size distribution, time-to-filter (TTF), sludge volume index (SVI) and supernatant turbidity, COD, biodegradability by incubation, and carbohydrate content by the phenol-sulfuric method.

This study is divided into five sections. In the second section theoretical background including activated sludge, as well as ultrasound and its basis as a treatment method are explored. Then the effects of ultrasound on different sludge characteristics are reviewed. In the third section material and methods of the study are presented. Results and discussion of the ultrasonication experiments are viewed in the fourth section of this study, which includes the analysis of the changes in sludge characteristics that were introduced in the theoretical background. The fifth and final section combines together all the previous information as conclusions. Some deductions and recommendations are also made in the last section.

2 Theoretical background

2.1 Activated sludge

2.1.1 The composition of activated sludge

Activated sludge is produced in the wastewater treatment process during the aerobic phase in the biological treatment. Activated sludge circles in the purifying process and excess activated sludge is removed from the cycle. Excess sludge is treated for disposal or for use. Activated sludge has a complex physical and chemical structure. It consists of minerals such as inorganic particles, and organic pollutants, multivalent cations, micro-organisms, and EPS. Activated sludge flocs are altogether negatively charged. (Urbain et al. 1993; Jin et al. 2004; Subramanian et al. 2010.)

The composition of sludge depends on the wastewater composition and the operational conditions of wastewater treatment, such as sludge age. The operational conditions affect the size, micro-structure, surface properties and density of activated sludge. Sludge flocs are comprised of small primary particles (≈ 2 μm), microflocs and compact flocculi (≈ 13 μm), and porous flocs (≈ 100 μm). Water occupies the biggest share of microbial aggregates. EPS are the second biggest component, followed by biomass. (Urbain et al. 1993; Chu et al. 2001; Jin et al. 2004; Subramanian et al. 2010.)

Activated sludge has the tendency to rather bind water than release it during the dewatering process in sludge treatment. The water content in sludge is generally separated into two categories, free water and bound water. Free water is the part that can be easily removed by thickening or by other weak mechanical manners. It behaves thermodynamically as pure water. The bound water is the portion that is tightly bound into the floc between sludge particles and cannot be removed by mechanical manners. The bound water has different chemical potential compared to free water and thus it acts thermodynamically in a somewhat different manner. (Urbain et al. 1993; Jin et al. 2004; Subramanian et al. 2010.)

The aqueous phase in sludge can be divided into four more categories; free water, interstitial water, surface moisture and bound moisture. Free water is water that is not bound to the sludge solids and can be easily separated. Interstitial water is trapped inside the sludge flocs. Interstitial water can be removed by the means of breaking floc structure. Surface moisture is attached by adsorption and adhesion on to the surface of the solid particles in sludge and cannot be removed by mechanical means. Bound moisture is

chemically bound to the sludge solids. The bound moisture is the hardest to remove but it can be done by thermo-chemical destruction of the particles. (Tsang & Vesilind 1990; Yin et al. 2004.) Physically bound water is attached by capillary forces while chemically bound water is attached by chemical bonds. Free water and physically bound water can be removed by mechanical means. Chemically bound water can be removed only by thermal drying at temperatures exceeding 105°C. Most of the water in sludge is bound by the EPS, having water content up to 98%. (Jin et al. 2004.)

EPS are biopolymers that originate from microbial activity. The EPS matrix is a gel-like environment which composition, structure and properties may vary dynamically as the micro-organisms vary and grow. EPS are produced by both eukaryotic (algae, fungi) and prokaryotic (Bacteria, Archaea) micro-organisms during either metabolism or lysis. EPS can keep the exoenzymes produced by bacteria near the cell surfaces and help the bacteria attach itself on surfaces. EPS may also bind material from outside the EPS matrix due to its adhesive properties. Foreign particles may integrate with the matrix influencing the microenvironment and the nutrient balance. EPS can bind metals such as nickel, copper, zinc, cadmium, and uranium or chemical substances such as benzene, toluene and xylene, or even grains of sand. EPS may originate also from wastewater itself containing e.g. cellulose and humic acids, or substances dissolved in water. EPS are somewhat soluble in water and some of the EPS in microbial aggregates are continuously dissolved in the water phase, contributing e.g. to COD. (Urbain et al. 1993; Dignac et al. 1998; Flemming & Wingender 2001a; Jin et al. 2003; Subramanian et al. 2010.)

EPS keep together microbial aggregates such as sludge flocs. They consist of proteins, polysaccharides, lipids, nucleic acids and biopolymers. The main portion of EPS are proteins with a share of 65 % of the total organic carbon. Glucose has the biggest share in total sludge sugar composition and it is also the main component of sugars in EPS, occupying around 34 % of the total sugars in sludge. (Urbain et al. 1993; Dignac et al. 1998; Flemming & Wingender 2001a.)

Operational parameters of wastewater treatment, sludge characteristics, dominating microbial community and the biochemical characteristics of EPS contribute to the concentration of EPS. EPS and their concentration in turn affect the thickening and dewatering properties of activated sludge. If the forces responsible for the adhesion of EPS matrix are exceeded, the matrix can be detached and broken down influencing the characteristics of sludge. The EPS concentration can be reduced e.g. by coagulation or through biological decay. (Novak et al. 1977; Flemming & Wingender 2001a; Subramanian et al. 2010.)

2.1.2 Activated sludge biodegradability, treatment and disposal

The excess sludge from wastewater treatment process is treated by aerobic or anaerobic digestion before utilization or disposal. The digestion process stabilizes and reduces sludge volume in order to ensure safe handling of excess sludge. As the micro-organisms consume biodegradable material, aerobic digestion produces carbon dioxide (CO_2) and anaerobic digestion produces biogas (methane, CH_4). The hydrolysis of cells and EPS are the rate limiting steps, limiting the rate and extent of both aerobic and anaerobic degradation. Anaerobic sludge digestion has four stages which are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The degradation degree of organic matter in anaerobic digestion varies between 25 to 60 % and secondary sludge from wastewater treatment is more readily degradable than primary sludge (Gröönroos et al. 2005; Yu et al. 2008.)

The most common sludge disposal methods in the European Union are agricultural use, composting and incineration (Figure 2.1). The use in land filling is decreasing as the European Union Directive 86/278/EEC is encouraging the use of sludge in agriculture. Excess sludge from the wastewater treatment process can also be utilized as raw material for commercially viable products such as biopesticides, biofertilizers, bioplastic and enzymes. These utilization methods are still novel, but are studied and developed. Bioconversion is considered more environmentally and economically friendly than conventional sludge disposal such as incineration or land filling. Wastewater sludge has many disadvantages in the bioconversion process, because it is not soluble, it has low homogeneity and it contains organic contaminants. The complex organic materials that wastewater sludge comprises of are not readily biodegradable and therefore the use of wastewater sludge as such is difficult. (Pham et al. 2009; European Commission 2015.)

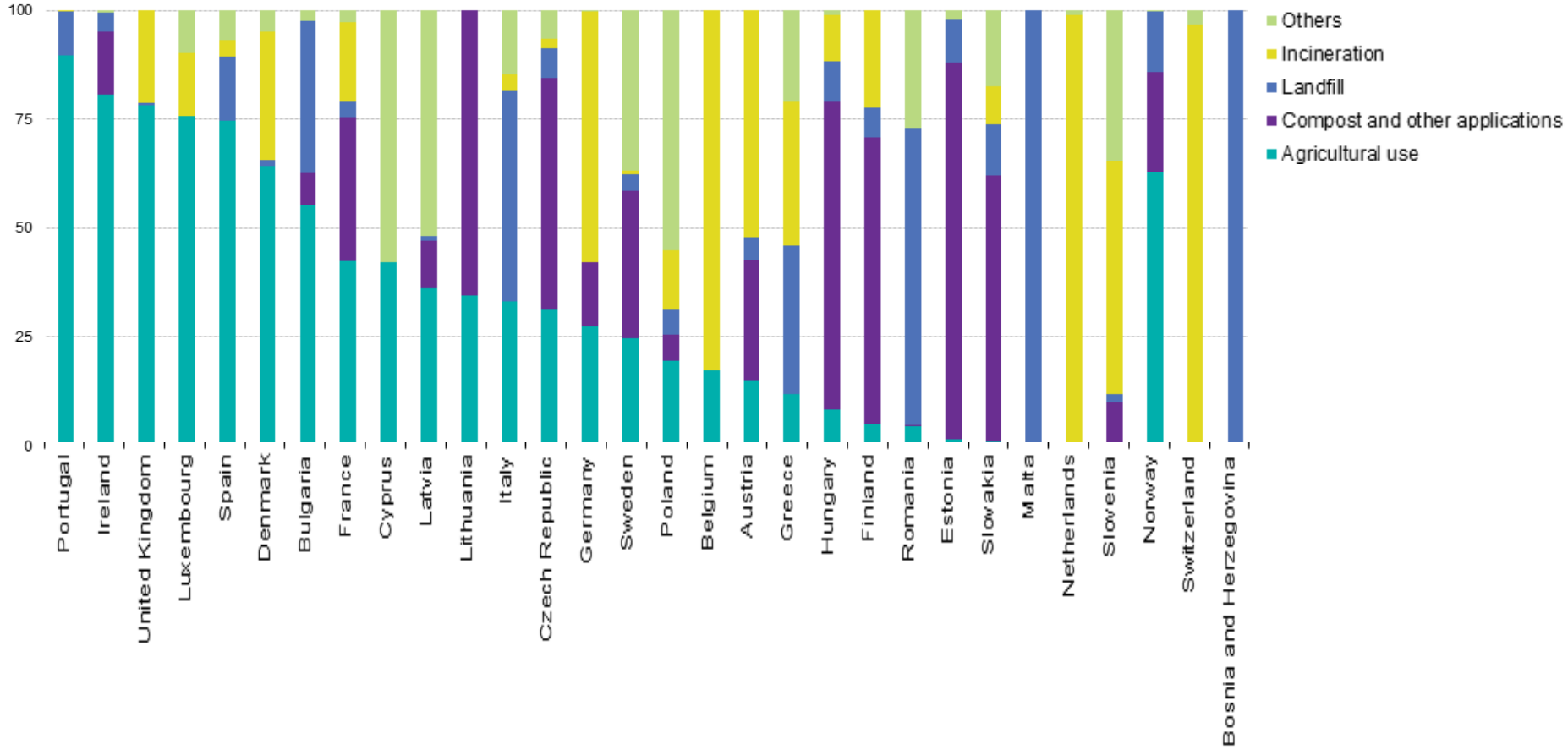

Figure 2.1. *Sewage sludge disposal from urban wastewater treatment by treatment type in 2013(% of total mass) in the European Union. Belgium, Denmark, Greece, Spain, Cyprus, Lithuania, Luxemburg, the Netherlands, Austria, Portugal, Finland, Sweden, and the United Kingdom; 2012.Croatia: data not available. (Eurostat 2015.)*

The anaerobic digestion is most commonly used method to stabilize sludge. Anaerobic digestion is relatively slow process and usually large fermenters are needed. The associated capital and operational costs of sludge treatment and disposal may be as high as 50% of the total costs of the entire wastewater treatment process. Pretreatment can accelerate hydrolysis and increase the degradation degree. Biodegradability of activated sludge and the rate of the process can be enhanced by using different pretreatment methods. These methods are ultrasound, mechanical disintegration, high pressure, alkaline addition, ozonation, thermal energy or the use of enzymes. Some methods can be combined to increase disintegration rates. The pretreatment processes rupture cell walls and membranes, release intracellular organics, and produce more biodegradable material for digestion. The advantage of using ultrasound is that it doesn't have any adverse effects on the environment, but it eases the biodegradation, decreases sludge viscosity and increases homogeneity. (Chu et al. 2001; Mao et al. 2004; Zhang et al. 2007; Pham et al. 2009.)

2.2 Ultrasonic treatment

2.2.1 Physical characteristics

The human hearing can detect sound frequencies from around 16 Hz to 18 kHz. Frequencies above 18 kHz are considered to be ultrasonic frequencies. Ultrasound is often divided into different categories based on its use. The categories are power ultrasound, range for processing and diagnostic ultrasound. The conventional power ultrasound ranges between 20 to 100 kHz, the range for processing is between 20 kHz to 1 MHz, and the diagnostic range is from 5 to 10 MHz. (Mason & Pétrier 2004; Parsons 2004.)

The medium used in ultrasonic sonochemistry applications can be divided into three categories according to their physical properties. These types are homogenous liquid, heterogeneous solid/liquid and heterogeneous liquid/liquid. Activated sludge is classified as a heterogeneous solid/liquid medium. (Mason & Pétrier, 2004; Parsons 2004.)

Ultrasound is induced into the medium through a transducer, which supplies vibrational energy. There are three different types of transducers, which are either fluid driven (liquid whistle), or electromechanically driven (magnetostrictive or piezoelectric). Ultrasound is lead into the medium by dipping the tip of the transducer into the medium or the transducer may be attached onto the bottom of the treatment vessel. The transducer generates energy which in turn causes the liquid to gain kinesthetic energy through ultrasonic wave. The applied energy is spent on cavitation, attenuation, adsorption and dissipation. The energy may cause disintegration and heating. The effect which ultrasound induces in the medium depends on the properties of the medium and the energy applied. (Parsons 2004; Show et al. 2007.)

The ultrasonic wave propagates through the medium as a longitudinal wave, creating positive pressures (compressions) and negative pressures (rarefactions) (Figure 2.2). The propagation of the ultrasonic wave can be somewhat altered by mixing the medium or applying a sufficient flow through the ultrasonic treatment. (Riesz et al. 1985; Mason & Pétrier 2004; Parsons 2004; Gröönroos et al. 2005.)

2.2.2 Cavitation

Cavitation means the formation and action of cavities in a liquid. Cavitation is produced when the vibrational energy overcomes the forces between molecules. Cavities appear as bubbles. In heterogeneous liquid/solid medium such as activated sludge, cavity bubbles may be trapped into the liquid boundary surface, in the interface of liquid and sludge particles. The cavitation phenomenon depends on the ultrasonic frequency and power. (Riesz et al. 1985; Laborde et al. 1998; Chu et al. 2001.)

There exists a critical power level below which ultrasound doesn't have an effect on the medium. Above the critical threshold pressure which the ultrasonic power generates, forces that bind particles together are exceeded and ultrasonic pressure affects the medium. Cavitation threshold pressure depends on the liquid at hand, the temperature of the medium, equilibrium of gas concentration, liquid surface tension, and the static pressure of the surrounding atmosphere. Lower surface tension gives a significantly higher cavitation threshold. If the cavitation threshold pressure is exceeded a minute cavity bubble will grow in the rarefactions. Gas from the medium enters the bubble and the bubble begins to expand during negative pressure sections. The gas composition inside the bubble may consist of gas, vapor or their mixture. The cavitation bubbles grow until they reach an equilibrium size, which depends on the applied sound frequency (Figure 2.2). (Riesz et al. 1985; Laborde et al. 1998; Mason & Pétrier 2004; Show et al. 2007.)

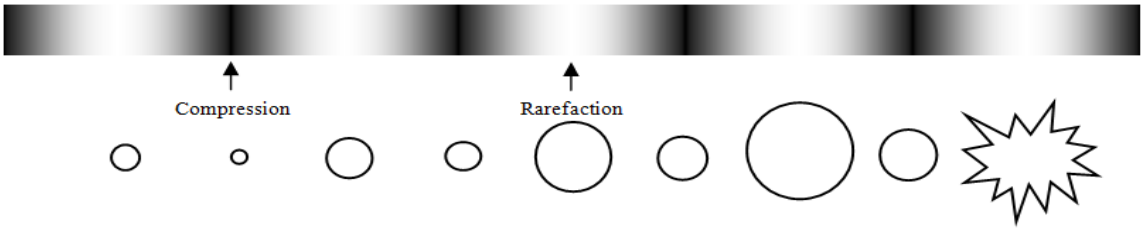

Figure 2.2. *Ultrasonic wave and cavitational bubbles (Parsons 2004).*

The cavitational bubbles may stay in stable cavities, which oscillate during thousands of acoustic pressure cycles (0.25s). Transient cavities exist for less than one cycle (0.002s). As either the stable or transient bubbles expand they gain potential energy due to the compressibility of the gas inside the cavity. Higher acoustic pressure is related to larger number of transient bubbles, which affect the medium more vigorously than stable bubbles. As transient bubbles are created and destroyed within a few microseconds, the

effect of them is more predominant compared to longer lasting transient bubbles in longer ultrasonication time. (Show et al. 2007.)

The oscillating bubble diameter in stable bubbles chances non-linearly and the radius of the cavitation is not proportional to the sound pressure. The size of the resonating bubble is inversely proportional to the applied frequency of the ultrasonic wave. For this reason, the cavitational bubbles collapse more violently at low frequencies (20 kHz) than at higher frequencies (500 kHz). (Riesz et al. 1985; Laborde et al. 1998; Chu et al. 2001; Parsons 2004.)

When cavitational bubbles collapse, they release kinetic energy. The kinetic energy produces very high pressures and temperatures. When the cavities collapse, the velocity of the liquid/gas interface approaches the speed of sound generating strong turbulent vortexes. Vortexes with diameter ranging from 5 to 100 µm are induced around the collapsing bubbles. The implosion of cavitation may produce temperatures higher than 3000 °K (≈ 2700 °C) and pressures over 1 000 bars. Bubbles may attract each other forming bubble clouds. The bubble clouds collapse from inside out, the preceding collapses strengthening the latter ones. The larger the bubble cloud is, the more violent is the implosion with a greater erosive effect. (Riesz et al. 1985; Laborde et al. 1998; Chu et al. 2001; Parsons 2004.)

If a precise frequency and intensity of ultrasound is combined with suitable geometry of treatment vessel, a standing wave can be accomplished which reinforces the cavitation. If the ultrasonication intensity is too high, standing waves are destructed by the cavitational activity. The amplitude of the standing wave depends on the liquid level. Biggest amplitudes are reached at odd multiples of the fourth of the wavelength and weakest amplitudes occur with even multiples of the fourth of the wavelength. (Riesz et al. 1985; Laborde et al. 1998; Dewil et al. 2006.)

The use of ultrasound in sludge treatment is usually based on cavitation. The cavitation effect can only be generated in a liquid medium, but it also affects particles in liquid. The most dominant ultrasonic frequency range in sludge treatment is between 20 to 40 kHz, which is in the range of power ultrasound. This frequency range generates cavitation most efficiently and thus the effects of ultrasonication are the greatest. Above 1 MHz the cavitation phenomenon doesn’t occur. (Chu et al. 2001; Mason & Pétrier 2004; Dewil et al. 2006.)

The implosion of cavitational bubbles can erode solids, initiate chemical reactions and even produce luminescence. In activated sludge imploding bubbles can disturb the structure of sludge flocs and EPS. Transient cavitation may also generate hydrogen atoms and hydroxyl radicals, which can form hydrogen and hydrogen peroxide, or react with the solutes in aqueous solutions. (Riesz et al. 1985; Laborde et al. 1998; Chu et al. 2001.)

2.2.3 Oxidative properties

In addition to the cavitational effects, ultrasound may also induce oxidative properties in aqueous solutions. The dissociation of water occurs through the thermal energy generated by the implosion of cavitation bubbles, producing highly active H and OH radicals. If the medium is saturated with oxygen, hydrogen peroxide may be produced from water and oxygen atoms. The formation of hydrogen peroxide may derive from the reactions between water, hydrogen atoms, oxygen atoms and molecular oxygen by following mechanisms (Mason & Pétrier 2004):

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \quad (2.1)$$

$$2HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad (2.2)$$

$$O_2 \rightarrow O + O \quad (2.3)$$

$$O + H_2O \rightarrow H_2O_2 \quad (2.4)$$

$$OH^{\bullet} + OH^{\bullet} \rightarrow HOOH \quad (2.5)$$

$$O + H_2O \rightarrow OH^{\bullet}OH^{\bullet} \quad (2.6)$$

Free radicals may induce chemical reactions within the collapsing bubble, at the interface of the bubble and the liquid, and in the vicinity of the bubble. Ultrasound may also generate nitrous and nitrate ions by reactions between hydrogen and hydroxyl radicals, but these ions are not considered to be a major participant in organic oxidations induced in sonochemistry. (Riesz et al. 1985; Laborde et al. 1998; Mason & Pétrier 2004; Parsons 2004.)

2.2.4 Ultrasonic treatment characterization

The acoustic pressure in ultrasonication depends on the properties of the medium and the properties of the applied ultrasound. Ultrasonic treatment can be characterized with many different expressions, which describe the relation between ultrasonic power and the medium, in this case sludge. Ultrasonic treatment variables are the energy, frequency and the duration of the applied ultrasound, and the quality of the sludge. High ultrasonic intensity leads to better disruption of cavitations due to the amplified acoustic pressure, which in turn overcomes more extensively the forces between sludge particles.

Acoustic pressure increases with the intensity of the ultrasonication, being proportional to the square foot of ultrasonication intensity. The efficiency of ultrasound decreases with input power at long distances from the transducer. High ultrasonic power as high specific energy is more efficient and disintegrates sludge more efficiently than lower power especially in the vicinity of the transducer. (Gröönroos et al. 2005; Show et al. 2007.)

The energy that is used for ultrasonication of sludge can be defined as specific energy (E_s). E_s is determined with the following equation:

$$E_s = \frac{P \cdot t}{V \cdot TS_0} \qquad (2.7)$$

Ultrasonic dose is expressed as:

$$UD_o = \frac{P \cdot t}{V} \qquad (2.8)$$

Ultrasonic dose can also be determined as:

$$UD = \frac{P}{V} \qquad (2.9)$$

Ultrasonic intensity is defined as:

$$UI = \frac{P}{A} \qquad (3.0)$$

where P is ultrasonic power (kW), t is ultrasonication time (s), V is sample volume (L), TS_0 is the initial concentration of TS (kg/L), and A is the surface area of the transducer (cm^2). (Show et al. 2007; Feng et al. 2009a; Pilli et al. 2011.)

Limiting factors in ultrasonic sonochemistry are e.g. reactor size, transducer type, the viscosity, the temperature and the TS content of the medium. Other contributors to the effects are container geometry and the position of the ultrasonic probe. (Chu et al. 2001; Gröönroos et al. 2005; Show et al. 2007.)

2.2.5 Operation optimization

The use of ultrasound as a pretreatment method is an energy-intensive process and its cost-effective use is a key concern along with its desirable effects. The use of ultrasound has high operational costs because of high capital costs and high use of electrical energy. The energy efficiency of ultrasonic transducers are poor and only 34 % of the electrical energy can be transformed into desired final effect. Usually a large amount of electrical energy is used to produce cavitation in small reactors and reaction volumes. (Show et al. 2007; Mahamuni et al. 2010.)

The optimization of energy use is important in ultrasonic applications and the form of propagation of ultrasonic wave is important to optimize in each case of ultrasonication of sludge, especially when designing a reactor scale-up. When ultrasonic reactors are designed, the input power and wave propagation along with other parameters should be carefully optimized to maximize positive effects and minimize negative effects. When designing ultrasonic reactors it is important to understand how the ultrasonic wave propagates through the medium. A high ultrasonic power and the vicinity of the cavitation near transducer may cause erosion on the transducer tip. (Chu et al. 2001; Gröönroos et al. 2005; Show et al. 2007.)

The most important parameter in optimizing operation is the proportion of ultrasonicated sludge, which contributes to the energy consumption, thus affecting strongly to the energy efficiency. TS concentration has a characteristic maximum value, which still enables the propagation of ultrasonic wave. Reactor size and shape, transducer type and specific energy along with the concentration of added polymer, limit the maximum value of TS content. (Gröönroos et al. 2005; He et al. 2011.)

If activated sludge has a very high solids concentration, the ultrasonic energy doesn't absorb into the sludge as efficiently as it would into a less concentrated sludge. At higher solids concentration the viscosity is reduced, reducing also the cavitation process since the ultrasonic waves are scattered and energy is absorbed more efficiently into the sludge closer to the transducer. As the energy absorption becomes more efficient, the temperature of the sludge raises. Micro-organisms may be inhibited due to higher substrate concentrations in high TS concentration. High solids concentration limits mass and oxygen transfer when pretreated sludge is digested. Both the ultrasound and the rise of temperature (due to the use of ultrasound) contribute to treatment efficiency. (Chu et al. 2001; Mao et al. 2004; Dewil et al. 2006; Pham et al. 2009.)

2.3 The effect of ultrasonic treatment on sludge characteristics

2.3.1 General effects of ultrasonic treatment

The use of ultrasound is being actively studied and developed for environmental applications. It can be used to remove surface contamination and biofilms, or to remove organic and inorganic contamination. It can also be used to control air-borne contamination. Biologically or chemically decontaminated water can be treated with ultrasound to break-up bacterial clumps, enhance cell rupture, increase cell permeability, or oxidate chemical and pesticide residues in combination with other treatment methods. In the treatment of sewage sludge, ultrasound has been used to defoam liquids and to stabilize and dewater sludge. (Parsons 2004.)

The treatment of activated sludge with ultrasound has been studied intensively to find out if it has positive effects in wastewater treatment. Factors studied are for example reduced activated sludge quantities, better dewaterability, the release of COD from solid to soluble form, and also the possible destruction of filamentous micro-organisms, which can cause bulking, a common and a very unwanted quality of sludge. (Chu et al. 2001; Mason & Pétrier, 2004; Parsons 2004; Zhang et al, 2007; Show et al. 2009.)

The application of power ultrasound (20-40 kHz) with sufficient power input into sludge induces cavitation, which in turn generates the destructive effects. Active radicals may also contribute to the effect. As the cavitation bubbles implode they produce jet streams. The jet streams cause mechanical shear forces which disintegrate sludge particles and induce dispersion of the suspended medium. Colliding particles also lead to erosion, surface cleaning and wetting of particles. Ultrasound can also lead to degassing as stable bubbles grow and float to the surface of the medium. Surface imperfections or gas trapped into the liquid can act as nuclei on which cavitation bubbles are formed more readily. Each sludge type from different wastewater treatment processes possesses different kind of qualities and therefore the cavitation threshold pressure varies for each sludge type. (Chu et al. 2001; Mason & Pétrier 2004; Parsons 2004; Zhang et al. 2007; Show et al. 2009.)

Low frequency and high intensity ultrasonic treatments are particularly good for disrupting sludge flocs and destroying cell walls and membranes, causing microbial cell lysis (Zhang et al. 2007; Pham et al. 2010). The use of ultrasound disintegrates sludge flocks releasing the EPS to achieve better hydrolysis, thus improving the subsequent aerobic and anaerobic digestion. (Gröönroos et al. 2005; Dewil et al. 2006; Yu et al. 2008; Xie et al. 2009.)

The effects of ultrasonic treatment can be divided into two stages. In the first stage (0-20min) the structure of sludge flocs are effectively disintegrated as mechanical shear forces break down porous flocs into small particles. In the second stage (20-60 min) some of the insoluble organic matter is transformed into a soluble state and biomass inactivates due to cell lysis. Excess energy is absorbed and causes the sludge temperature to rise. (Chu et al. 2001; Pham et al. 2009.)

The positive effect of low-intensity ultrasound on biotechnological processes has been acknowledged by several researchers. In addition to sludge floc and particle disintegration, ultrasound can also enhance enzyme activity, cell membrane permeability, cell growth and biosynthesis. It has also been suggested that low-intensity ultrasound could be applied directly to wastewater treatment process to stimulate biological activity and hence increase the removal of pollutants, and nitrogen and phosphorous. (Pitt & Ross 2003; Xie et al. 2008; Rokhina et al. 2009; Xie et al. 2009; Mahamuni et al. 2010.)

2.3.2 The effect of ultrasound on temperature and pH

The excess power that doesn't break any ponds in the sludge heats it. This is especially remarkable with long treatment times and has to be taken into account. The higher the dry solids content is, the higher the temperature rise. This happens because sludge density enhances cavitation and more energy can be absorbed. Cavitation produces turbulent movement into the sludge guaranteeing a good heat transfer throughout the ultrasonication vessel. Disintegration of sludge is enhanced with treatment temperature, and it also increases the amount of soluble COD (SCOD) when the ultrasonic treatment has a heating effect on sludge. (Chu et al. 2001; Gröönroos et al. 2005; Dewil et al. 2006.)

Without cooling, the temperature of sludge increases distinctly with ultrasonication. With long irradiation times the temperature of sludge can increase from 5 °C to higher than 60 °C. Rising temperature has an influence on the release of COD into soluble state. When the rise in temperature is prevented with a sufficient cooling, it can be found that ultrasound treatment alone can transform only a definite amount of total COD (TCOD) into SCOD. Whereas combined with heating, COD is continuously released to supernatant during ultrasonic treatment time. On the other hand, heating alone can't disintegrate floc structure, but it does cause cell lysis leading to increase in SCOD. (Chu et al. 2001.)

Excess temperatures should be avoided if activated sludge is to be recycled back to the biological process. On excess sludge, heating generally has a positive effect. Ultrasonic treatment doesn't have a remarkable effect on the pH of activated sludge. (Dewil et al. 2006; Zhang et al. 2007.)

2.3.3 The effect of ultrasound on sludge settling and dewaterability

Separation of water from biological solids is a key operation in wastewater treatment and activated sludge systems. The dewatering process results in sludge volume reduction, which makes it easier to transport, handle, store, utilize and dispose. The dewatering process is a compromise between efficiency and economical feasibility. Better dewatering requires more energy, which in turn signifies more costs. The dewatering process is one of the most costly operations in wastewater treatment and it is also where most of the operational problems and process failures occur. (Novac et al. 1977; Jin et al. 2004.)

Floc characteristics, particularly the size and presence of particles, affect the dewaterability. High values of flocculating ability, hydrophobicity, negative surface charge and viscosity in activated sludge indicate poor dewaterability. The EPS in activated sludge act as flocculants and thus high concentrations of EPS may indicate a poor settling and dewaterability properties (Urbain et al. 1993; Jin et al. 2003). On the other

hand, high concentrations of Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} seem to improve the dewaterability significantly. When sludge is treated with ultrasound the dewaterability becomes more laborious. The more ultrasonic energy is applied, the harder the dewatering process becomes. This can be detected as the increase in capillary suction time (CST) and TTF. There is a strong correlation between decrease in sludge floc size and deterioration of sludge dewaterability. (Chu et al. 2001; Flemming & Wingender 2001b; Jin et al. 2004; Dewil et al. 2006.)

When sludge flocs disintegrate, the amount of micro-particles increase in the supernatant of settled sludge. At high energy doses, the release of micro-particles is more effective. The released micro-particles settle poorly since their density is almost equal to that of water, affecting the supernatant turbidity. (Feng et al. 2009b.) Small particles also provide a large surface area where water can be attached during e.g. filtration. Small particles cause also the filtration cake to clog more easily affecting negatively the filterability. (Chu et al. 2001; Dewil et al. 2006.)

Activated sludge settling characteristics are considered to be acceptable when SVI is at least 100 ml/g. (Dewil et al. 2006.) When high energy ultrasound is applied, sludge settleability diminishes. The difference in settling velocity between ultrasonically treated and untreated sludge may reflect the differences in the size and shape of flocs resulting from ultrasonication. After ultrasonication a re-flocculation phenomenon may be observed. This phenomenon affects the settling velocity and is occurs due to microbiological activity and the release of intra- and extracellular biodegradable matter. (Feng et al. 2009b.)

2.3.4 The effect of ultrasound on particle sizes

High-intensity ultrasound affects the size of sludge particles. The sizes of sludge particles decrease significantly as soon as ultrasound is applied. Within the first five minutes of ultrasonication, particle sizes decrease most significantly. After that, ultrasonication continues to decrease sludge particle size. With longer ultrasonic treatment times, a re-flocculation phenomenon may also be observed. Re-flocculation is detected as an increase in particle sizes and it depends on sludge characteristics and activity. (Chu et al. 2001; Gonze et al. 2003; Mao et al. 2004; Bougrier et al. 2005; Dewil et al. 2006; Feng et al. 2009b.)

The ultrasonic irradiation time and specific energy input affect the sludge floc particle sizes. The particle size of sludge and the specific energy dosage of ultrasound are inversely related. As irradiation time and specific energy input increase the floc sizes decrease. The decrease in particle sizes leads to deteriorated dewaterability. (Chu et al. 2001; Dewil et al. 2006; Feng et al. 2009b.)

2.3.5 The effect of ultrasound on solids and COD

As sludge is being treated with ultrasound, a continuous sludge disintegration and mass reduction can be observed as a reduction in SS and VS. The decrease of VS leads to the increasing value of organics, such as proteins and nucleic acids, in supernatant after settling of sludge. This is due to floc disintegration and cell lysis, which releases intracellular material. The difference between SS and VS concentration indicate the presence of inorganic compounds. (Gonze et al. 2003; Zhang et al. 2007.)

The reduction of VS could be used as an index for floc disintegration. The difference between SS and VS may be regarded as the concentration of inorganic compounds in the sludge. The inorganic matter stays stable during ultrasonication. The sludge mass reduces due to the transformation of organic matter into the liquid phase. (Zhang et al. 2007.)

Ultrasound affects the COD in activated sludge changing the ratios of COD in solid and soluble state (SCOD/TCOD). Studies have shown that depending on the composition of activated sludge the major part of TCOD is biodegradable. Most of the TCOD in untreated sludge may be associated with the solid phase, rather than in soluble form which can be analyzed as SCOD. As sludge is treated with ultrasound the SCOD/TCOD ratio ascends suggesting that the COD is transformed into soluble form into sludge supernatant. The longer the irradiation time, the more TCOD is transformed into SCOD. The increasing SCOD during ultrasonication is due to the release of intra- and EPS during both floc disintegration and cell lysis. The destruction of COD depends not only on the irradiation time, but also on the specific energy input of the ultrasonic transducer and sludge temperature. (Chu et al. 2001; Dewil et al. 2006; Zhang et al. 2007; Pham et al. 2009; Feng et al. 2009b.)

The efficiency of ultrasonic treatment increases as TS content increases. The efficiency is noticed as an increase in the SCOD even though equal amount of relative energy is applied. This phenomenon has a maximum value of dry solids content, above which the efficiency starts to decrease. The decrease is attributed to the increased viscosity, which prevents cavitation as ultrasonic waves are scattered and absorbed into the sludge. (Dewil et al. 2006.)

Even though the effect of ultrasound on sludge particle size diminishes significantly after the first period of ultrasonication (5 min), the COD continues to transform into soluble state. The higher concentrations of SCOD are reached, the more efficient can be expected the subsequent anaerobic digestion to be. (Mao et al. 2004.)

2.3.6 The effect of ultrasound on biodegradability and sludge activity

In the biological degradation process, micro-organisms consume biodegradable material that is available for them in certain conditions. From analytical point of view the amount of TS concentration is reduced. (Tiehm et al. 1997.)

SCOD attributes largely to the oxygen demand of biodegradable matter. If biological oxygen demand (BOD) and TCOD are being compared along ultrasonic treatment methods, varying time and irradiation energy, it can be said that as the BOD/TCOD ratio increases, it indicates that most of the released COD is biodegradable. Ultrasonic treatment therefore enhances sludge biodegradability. The release of COD may also be due to cell lysis and deterioration of micro-organisms. (Chu et al. 2001; Dewil et al. 2006; Feng et al. 2009b.)

The experiments with ultrasound on sludge imply that subsequent acidogenesis, acetogenesis and methanogenesis reactions in anaerobic treatment would be enhanced. These biological reactions in turn improve the biogas (methane) yield and reduction of sludge volumes. Ultrasonic pretreatment can reduce the anaerobic digestion time from 20 to 8 days increasing also the production of biogas potentially from 5% to 10%. (Onyeche et al. 2002; Dewil et al. 2006; Mao 2007; Zhang et al. 2007.)

Even with low ultrasonic power, the microbial density levels may be reduced after long ultrasonication times. The weakening of microbial activity may happen even if the floc size and dewaterability are not affected with ultrasonication. In some studies (Dewil et al. 2006) filamentous micro-organisms in the waste activated sludge were not decreased at low intensities and short term duration of the ultrasonicaton. Depending on the energy input and exposure time of ultrasound, and the TS content of the sludge, a strong cell lysis may occur. The decrease in microbial activity can be observed as a decrease in oxygen uptake rate (OUR). Not only cavitation, but also chemical reactions might contribute to the decrease of viability of sludge. (Chu et al. 2001; Dewil et al. 2006, Zhang et al. 2007.)

3 Materials and methods

3.1 Sample collection

The sludge used in this study was excess sludge (Table 3.1) from a municipal wastewater treatment plant located in the city of Murcia, Spain. After sampling the sludge was kept at 4 ± 1°C for 5-15 days before it was used in the experiments.

Table 3.1. *Characteristics of the excess sludge used in this study.*

TS	(g/l)	11.4
SS	(g/l)	10.4
VS	(g/l)	6.4
COD	(mg O_2/g TS)	4600
SCOD	(mg O_2/g TS)	38.0
COD	(mg O_2/g TS)	24.6

3.2 Experimental set up

The sludge was treated with a pulsating ultrasound with pulses that were five seconds long and with a two second break between each pulse. Total ultrasonication times (consisting of seven second periods, including pulse and break) were 5, 10, 20, 30 and 60 minutes. The ultrasonication device was a Misonix sonicator 3000 with an ultrasonic converter (S/N R1686). The maximum power of the ultrasound was 0.264 kW and amplitude of the ultrasound was 8.00.

For each ultrasonication duration time two 800 ml batches of sludge were treated to gain the amount of ultrasonically treated sludge that was needed for settling experiments (1000 ml) and other analysis. Ultrasonication took place in a glass vessel that was cooled by a water jacket. The tip of the ultrasonicator (sonotrode) was dipped into the sludge so that it was 25 mm off from the bottom of the treatment vessel (Figure 3.1). After the first ultrasonication for each irradiation time, around 250 ml of the ultrasonicated sludge was taken to analyses. After the two ultrasonications in the same irradiation time, both amounts of sludge were mixed and further analyzed.

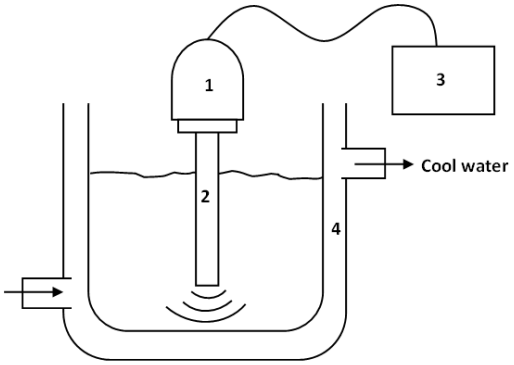

Figure 3.1. *Schematic diagram of the ultrasonication treatment reactor with the ultrasonic transducer (1), the sonotrode (2), the frequency generator (3), and the water jacket (4).*

Another set of ultrasonication experiments was performed for sludge stored at 4 °C for 60 days. The former and latter experiments are later on referred to as comprehensive and limited experimental set-ups respectively. Sludge was characterized by means of TS, supernatant turbidity, TCOD, and SCOD in both comprehensive and limited experimental set-ups. Also particle size distribution, VS, SS, TTF, settled sludge volume, SVI, COD_f and carbohydrate content were recorded in the comprehensive experimental set-up. Biodegradability was analyzed in the limited experimental set-up.

3.3 Analytical methods

3.3.1 Settling, SVI, supernatant turbidity and filterability

Settled sludge volumes were done according to standard methods (Tests on sludges 2710, 2012) in the comprehensive experimental set-up. Settled sludge volumes were measured by measuring 1000 ml of ultrasonicated sludge into a graduated cylinder. The sludge was mixed and was allowed to settle for 1 hour. The volumes of sludge sediments were read in the cylinder after 5, 10, 15, 20, 25, 30, 40 50 and 60 minutes. No stirring was used in the settling. Settled sludge volumes were determined as the height of sludge sediments after a certain fixed time interval.

SVIs were determined according to standard methods (Tests on sludges 2710, 2012) in the comprehensive experimental set-up. SVI is the volume in milliliters occupied by 1 g of a suspension after 30 minutes of settling. Thus by using the 30 minutes settled sludge volume that is gained in the settled sludge volume experiments and dividing it with the amount SS, the SVI is gained (equation 3.1).

$$SVI = \frac{settled\ sludge\ volume \frac{ml}{l} \times 1000}{suspended\ solids \frac{mg}{l}} \quad (3.1)$$

Supernatant turbidities were measured following the procedures of Gonze et al. 2003 in both comprehensive and limited experimental set-ups. The supernatant turbidities were measured to evaluate the quality of the supernatant and they were measured after 1 hour of settling with Aqualytic turbidity LAB-IR equipment at wavelength of 880 nm, which is in the infrared area.

Filterabilities were measured by recording the TTF (Tests on sludges 2710, 2012) in comprehensive experimental set-up. The test required 200 ml of sludge that was filtered through a Whatman no. 541 filter paper. A filtration system with a graduated cylinder and a stopwatch were needed. The sample was filtrated with a pressure of 59 kPa, and the time that it took to collect 100 ml of sample was recorded. Different vacuum pressure, filter support type, sludge temperature, and sample volume affect the test results. The filtration was carried out in a Buchner-filtration at 59 kPa vacuum and no polymers or polyelectrolytes were added before filtration.

3.3.2 Particle size distribution

The particle size distributions of sludge samples were measured in the comprehensive experimental set-up with a Malvern Mastersizer2000 with a Hydro2000 mixing unit using procedure described e.g. by Gonze et al. (2002). Samples were collected after ultrasonication and the 1 hour settling measurements, which followed the ultrasonication. Samples were collected with a wide bore pipette from the bottom of the settling vessel where the sedimented sludge lied. Each time approximately 1.5 ml of concentrated sludge was collected and the weight of the collected sample was measured. The pipette bore was cut to be 7.5 mm so that also the long strains in the sludge would get into the pipette. Before analysis the sludge was diluted 400-fold by carefully adding the 1.5 ml of sludge into 600 ml of water. Tap water was used for the background measurement as blank and for dilutions.

Each time before measuring samples, the background noise of measurement (only tap water) was measured to eliminate possible disturbance caused by the tap water used for dilution. The pump propeller speed was at 1350 rpm. After measuring the background the propeller mixing speed was reduced to 250 rpm to avoid the breakup of sludge particles. Samples were released into the water and they were allowed to mix with water for 15 seconds. After these procedures the particle size distribution was measured.

3.3.3 Solids and COD

The protocol from the standard methods was used as a guideline for the determination of TS, VS and SS (Solids 2540, 2012). The TS of the sludge samples were measured each time after ultrasonication in both comprehensive and limited experimental set-ups.

VS were measured after each ultrasonication and the determination of TS in the comprehensive experimental set-up, and the SS were determined by filtering the sample through a glass-fiber filter in the comprehensive experimental set-up. The filtrate was collected and used for the analysis of COD in filtrate (COD_f).

Samples for the TCOD were collected immediately after ultrasonication from a mixed sample. The SCOD were measured from sludge supernatant after ultrasonication and settling procedures and the COD_f were measured from ultrasonicated samples after filtration. The determination of TCOD, SCOD and COD_f were analyzed according to standard methods (Chemical oxygen demand 5220, 2012). The method used was the closed reflux titrimetric method. TCOD and SCOD were determined for each of the samples in the comprehensive and limited experimental set-ups. Also the filtrate of the SS determination was collected to analyze the COD_f in the comprehensive experimental set-up.

3.3.4 Biodegradability and carbohydrate content

Biodegradability of ultrasonicated sludge samples was determined in the limited experimental set-up according to method described by Pham et al. (2010). Sludge samples (75 ml) were inoculated in Erlenmeyer glasses in 25 ± 1 °C and 150 rpm on a rotary shaker for 18 days. A thermostat cabinet Aqualytic VELP scientific B.O.D sensor system 6 was used for the incubation. The biodegradability was then assessed by the decrease in TS consumed by the micro-organisms after the incubation (equation 3.2). Due to volume losses because of evaporation, the water level was adjusted again to the original volume of 75 ml with Milli-Q water.

$$Biodegradability = \left(1 - \frac{Total\ solids\ after\ biodegradation}{Total\ solids\ before\ biodegradation}\right) \times 100\ \% \qquad (3.2)$$

The carbohydrate content of ultrasonicated sludge samples was determined after the extraction of EPS in the comprehensive experimental set-up. EPS extraction was done according to the method by Subramanian et al. (2010). EPS extraction was carried out by means of centrifugation and precipitation with ethanol. After the extraction of EPS, the carbohydrate content was analyzed from the remaining supernatant by the method introduced by Dubois et al. (1956). The analysis of carbohydrate content was done by using glucose as a standard. The color of prepared samples was measured with a Biochrom Libra S22spectrophotometer at the wavelength of 490 nm. Blanks were prepared by substituting sugar solution with distilled water and all samples were made in duplicates.

4 Results and discussion

4.1 The effect of ultrasound on temperature and pH

The effect of ultrasound on sludge temperature and pH was assessed by measuring the sludge temperature before and after each ultrasonication while the pH was measured only after each ultrasonication (Table 4.1, Figure 4.1). Even with a cooling water jacket and a continuous water flow over the treatment vessel, sludge temperature raised and after five minutes of ultrasonication, the sludge temperature had risen more than 20 °C in both experimental set-ups. The sludge temperatures followed logarithmic trend lines which had positive correlations of 0.96 and 0.99 in the comprehensive and limited experiments, respectively.

Table 4.1. *The effect of ultrasound on sludge temperature and pH in the comprehensive and limited experimental set-ups.*

Comprehensive experimental set-up						
t_{US} (min)	0	5	10	20	30	60
temp1 (ºC)	4.4	4.3	4.0	3.3	4.4	3.4
temp2 (ºC)	4.4	28.7	30.6	31.2	35.9	44.3
Δtemp (ºC)	0.0	24.4	26.6	27.9	31.5	40.9
pH	7.4	7.3	7.2	7.2	7.4	7.4
Limited experimental set-up						
temp1 (ºC)	4.4	4.0	4.0	4.0	3.9	4.1
temp2 (ºC)	4.4	25.3	28.0	33.7	38.3	39.7
Δtemp (ºC)	0.0	21.3	24.0	29.7	34.4	35.6
pH	7.2	7.2	7.2	7.2	7.1	7.1

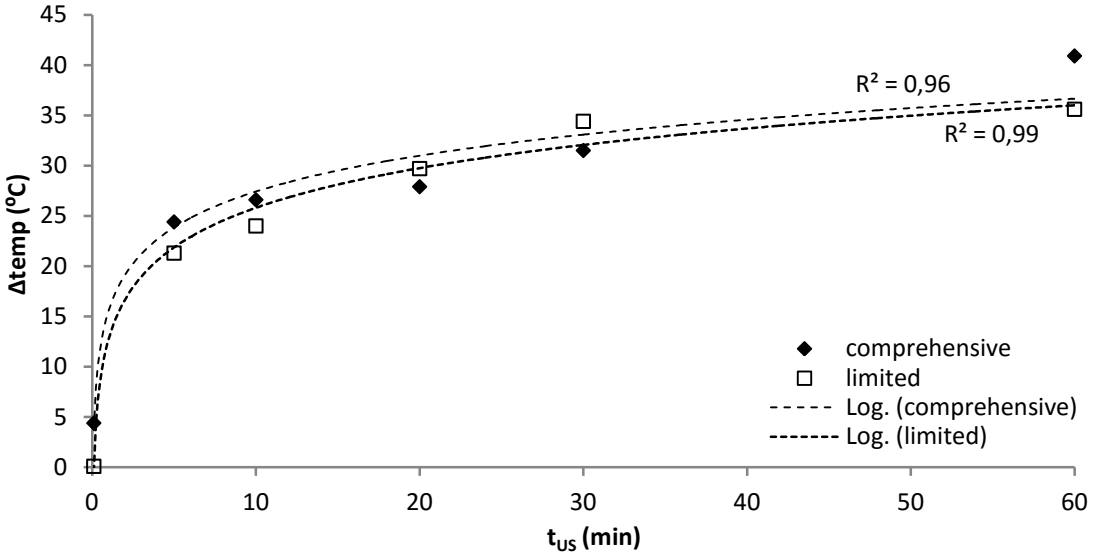

Figure 4.1. *The effect of ultrasound on sludge temperature in comprehensive and limited experimental set-ups.*

The effect of ultrasonic treatment operation conditions on sludge temperature were also monitored by both Chu et al. (2001) and Dewil et al. (2006). In both studies, ultrasonication time and ultrasonic power level were varied and the sludge TS was around 8.5 g/l. After 2 minutes of ultrasonic treatment Chu et al. (2001) reached a temperature difference of approximately 37 °C (at 0.44 W/ml), whereas Dewil et al. (2006) reached a temperature difference of approximately 4°C (at 0.43 W/ml). In this study the temperature difference after 5 minutes of ultrasonic treatment was around 23 °C (11.4 g/l, 0.33 W/ml). A strict comparison on the effects of ultrasonic treatment is not possible as it appears that the sample sizes seem to affect the results. The highest temperature rise (37 °C) was reported in study with a 250 ml sample volume (Chu et al. 2001), while the temperature rise was lowest (4 °C) with a 2300 ml sample volume (Dewil et al. 2006). In this study the temperature difference was between 24.4 °C and 40.9 °C (from 5 to 60 minutes of ultrasonication) with sample volume of 800 ml. Ultrasonication didn't have any effect on the pH of the sludge samples.

The most important variables that affect sludge temperature are ultrasonic treatment time, ultrasonic power level, sample size, and sludge solids content. The more the temperature rises, the more COD solubilized as discussed in Chapter 4.6. Rise in temperature may also affect the sludge activity, and at long ultrasonic treatment times and high temperatures, sludge may be inactivated as discussed in Chapter 4.7.

4.2 The effect of ultrasound on settling and filterability

The effect of ultrasound on sludge settling was determined in the comprehensive and limited experimental set-ups. The settled sludge volumes and SVIs were measured in the comprehensive experimental set-up (Tables 4.2 and 4.3, Figures 4.2 and 4.4) and the

sludge supernatant turbidities were measured in both comprehensive and limited experimental set-ups (Table 4.4 and Figure 4.5). In the limited experiments the supernatant turbidities were measured after the sludge was allowed to settle for 6 days in 4 °C.

The data for sludge settling shows clearly that without ultrasonication the sludge settles slowly. After 30 minutes of settling the sludge had settled only 70 ml. And after an hour of settling the sludge had settled a total of 175 ml. The settling of non-ultrasonicated sample indicated that the structure of the sludge was very loose. After ultrasonic treatment the sludge settling improved and sludge settled more rapidly, especially in the beginning of the settling process. The final volume of sludge sediments also decreased. The longer the ultrasonic pretreatment was, the more easily the sludge settled. The best settling characteristics were reached when sludge was ultrasonicated for 20 minutes or more.

Table 4.2. *The effect of ultrasound on the settled sludge volumes in the comprehensive experimental set-up.*

Settling time (min)	t_{US} (min)	0	5	10	20	30	60
0		1000	1000	1000	1000	1000	1000
5		990	950	910	290	90	60
10		980	890	800	250	60	50
15		965	820	720	230	50	47
20		952	770	660	220	47	44
25		940	720	620	210	44	42
30		930	690	590	200	43	40
40		900	630	540	190	40	38
50		865	590	500	190	40	36
60		825	560	480	190	40	34

The settling process was very similar after 30 and 60 minutes of ultrasonic treatment. After just 5 minutes of settling, both sludge samples had already nearly reached their final volumes. After 5 minutes of settling the sludges that were ultrasonicated for 30 and 60 minutes had settled a total of 910 ml and 940 ml respectively and the final volumes of settled sludge were 40 and 34 ml respectively. The settling rate increased with increasing ultrasonic dose also in the study by Gonze et al. (2003) where the settling rate increased from an initial rate of 0.50 to 2.40 m/h (0 to 156 kJ/l). The settling velocity increased constantly in all sludge samples in the study by Feng et al. (2009b) until one hour, after which settling velocity decreased.

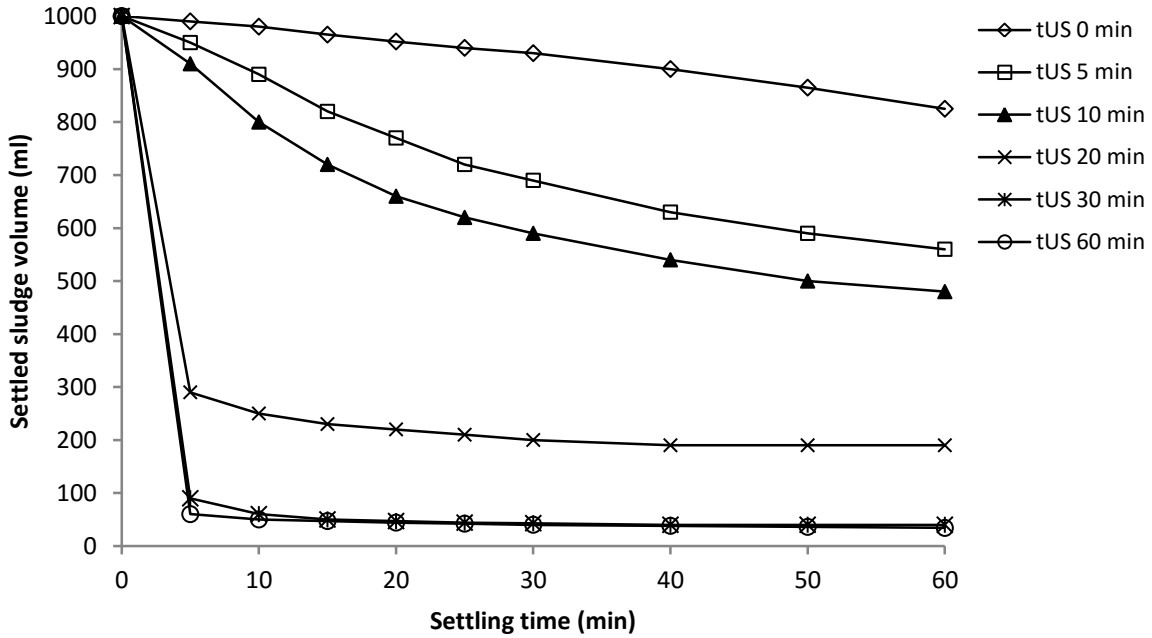

Figure 4.2. *The effect of ultrasound on the settled sludge volume in relation to settling time in the comprehensive experimental set-up.*

SVIs in the current study showed a constant decline (from 124 to 10 ml/g) as the ultrasonication time increased from 0 to 60 minutes. A logarithmic trend line depicted well the evolution of SVI during ultrasonic treatment (Figure 4.4). The logarithmic trend line reached a positive correlation of 0.97.

Table 4.3. *The effect of ultrasound on settled sludge volume after 30 minutes of settling, SS of sludge samples, and SVIs in the comprehensive experimental set-up.*

t_{US} (min)	0	5	10	20	30	60
Settled sludge volume (ml/l)	930	690	590	200	43	40
SS (mg/l)	7520	9340	9540	10400	3260	3960
SVI (ml/g)	124	74	62	19	13	10

The SVI decreased from 218 to 125 ml/g with ultrasonic doses from 0 to 156 kJ/l respectively in the study by Gonze et al. (2003) where both settling rate and SVIs improved. Dewil et al. (2006) gained a very different kind of results with a bulking sludge and short treatment times (10-75s). Their settling characteristics were worsened after treatment and the SVI could not be reduced with any combination of duration, amplitude and TS concentration of sludge. They observed a turbid supernatant and very loose settled layer. The SVI of non-ultrasonicated sludge was 151 ml/g and after 75s ultrasonic treatment (at 500W) the SVI was 344 ml/g (Dewil et al. 2006).

In the current study the supernatant turbidity increased rapidly after ultrasonic treatment was initiated in both comprehensive (Figure 4.3) and limited experimental set-ups. The turbidities in the comprehensive experiments change irregularly during treatment, vary-

ing from 1.62 NTU·l/g in the non-ultrasonicated sludge to 66.12 NTU·l/g in the sludge that was ultrasonicated for 5 minutes.

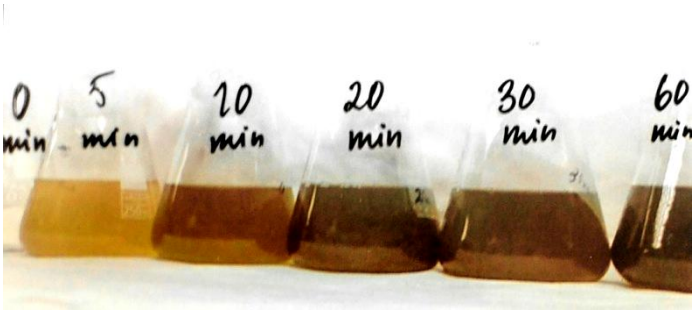

Figure 4.3. *Sludge supernatants which were extracted after ultrasonic treatment in the comprehensive experimental set-up.*

The irregular behavior may be due to an inconsistency in sampling or error in measurement or the fact that the sludge may have been more active and re-flocculation may have affected the turbidity. In the limited experiments the turbidities change more logically, increasing linearly from 0.51 NTU·l/g in the non-ultrasonicated sludge to 49.89 NTU·l/g in the sludge ultrasonicated for 10 minutes and staying at a fairly constant level afterwards (42.24-58.13 NTU·l/g).

Table 4.4. *The effect of ultrasound on sludge supernatant turbidities in the comprehensive and limited experimental set-ups.*

t_{US} (min)	0	5	10	20	30	60
Comprehensive experimental set-up						
Turbidity(NTU)	13.60	646	483	174	290	124
TS (g/l)	8.36	9.77	10.44	10.74	4.53	4.73
Turb./TS (NTU·l/g)	1.62	66.12	46.26	16.20	64.18	26.22
Limited experimental set-up						
Turbidity(NTU)	5.90	295	563	603	599	495
TS (g/l)	11.64	11.63	11.28	10.37	11.67	11.72
Turb./TS (NTU·l/g)	0.51	25.37	49.89	58.13	51.34	42.24

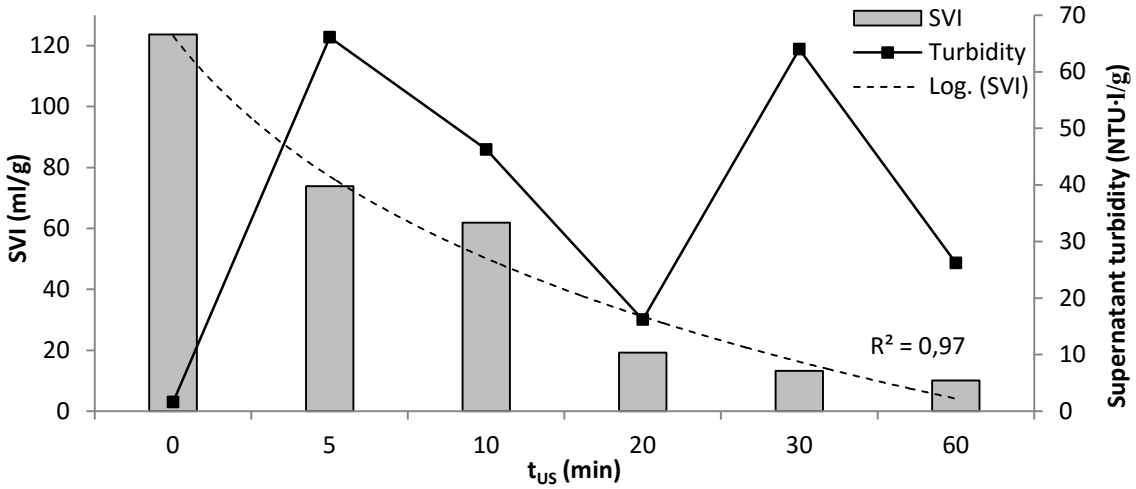

Figure 4.4. *The effect of ultrasound on turbidity and SVI in the comprehensive experimental set-up.*

Supernatant turbidity in relation to TS yielded different results in the comprehensive and limited experimental set-ups (Figure 4.5). The sludge samples in limited experiments settled for 6 days at 4°C after ultrasonication, allowing the smaller and more slowly settling particles to descend. On the other hand some particles may have caused re-flocculation and ascended due to microbial activity. This in turn increased the turbidity. The values supernatant turbidities are a combination of the phenomenon of the settling of particles and particles that ascend due to re-flocculation.

Feng et al. (2009b) used a settling period of 8 hours after which the supernatant turbidity was measured. They concluded that if E_s of less than 1000 kJ/kg is used, the supernatant turbidity decreases. With greater E_s, turbidity increases due to the increasing amount of micro-particles which were released from sludge flocs. The smallest turbidity in the study by Feng et al. (2009b) was measured at 5 NTU and biggest was measured at 60 NTU. Na et al. (2007) gained results where supernatant turbidity was between 35 and 370 NTU. From 0 to 400 kJ/l volumic energy doses, the turbidity increased significantly and the rise leveled off at energy doses from 400 to 1200 kJ/l.

Turbidity increases when the applied E_s increases (Tiehm et al. 2001; El-Hadj et al. 2007; Na et al. 2007). A threshold value for the E_s was discovered in the study by El-Hadj et al. (2007), where at E_s less than 5000 kJ/kg the turbidity of the sludge supernatant decreased and above it increased drastically. Bougrier et al. 2005, Feng et al. (2009b) and Gonze et al. (2003) all concluded that energy doses less than 1000kJ/kg did not increase the supernatant turbidity of sludge indicating that the minimum energy required to disrupt the sludge is 1000 kJ/kg. The differences between different studies are due to different sludge characteristics and operating conditions.

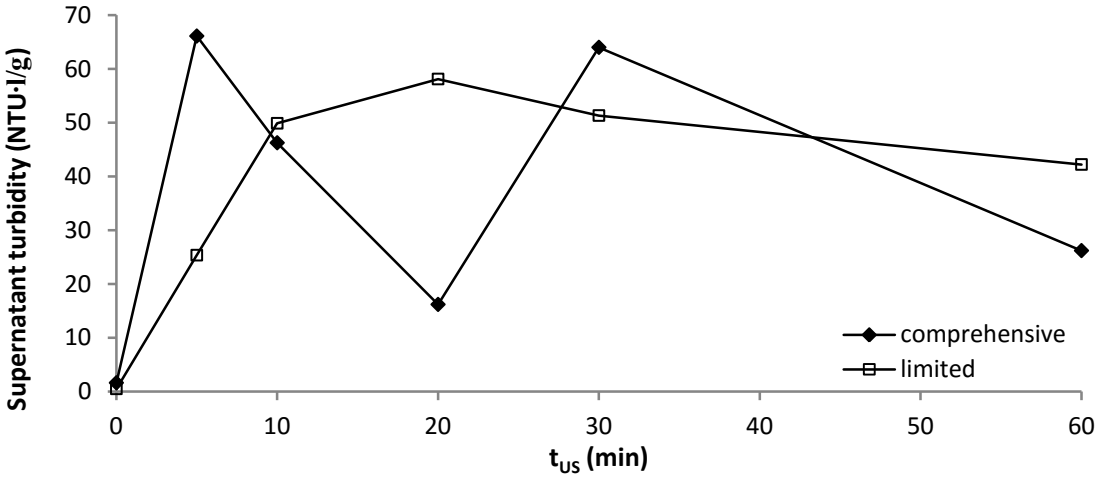

Figure 4.5. *The effect of ultrasound on supernatant turbidity in relation to TS in the comprehensive and limited experimental set-ups.*

The effect of ultrasound on sludge filterability was studied with non-ultrasonicated sludge and with sludge that was ultrasonicated for 5 minutes (Table 4.5). Samples were measured in duplicates. The longer ultrasonication times resulted in sludge samples that caused fast and excessive clogging of filter paper and thus TTF experiments were not carried out.

Table 4.5. *The effect of ultrasound on TTF.*

t_{US} (min)	0	5
TTF (s)	21	> 900
TTF (s)	16	>900

The present results and previous studies show that ultrasonication has a significant negative effect on sludge dewaterability. Particles in sludge provided a surface area, where the water in sludge attached. As ultrasonic treatment decreased the particle sizes, sludge surface area became larger and more water was attached onto the surface of sludge particles. The filtration cake became clogged blocking the flow through filtration paper. The CST of ultrasonicated sludge, which correlates with TTF, was reported by Chu et al. (2001), Gonze et al. (2003) Dewil et al. (2006), Na et al. (2007) and Feng et al. (2009a). Dewaterability deteriorated after ultrasonic treatment in the current study and in the abovementioned studies. Sludge filterability decreased with increasing ultrasonication time and E_s (Chu et al. 2001, Dewil et al. 2006).

Ultrasonication treatment volumes and TS influence the degree dewaterability. Low E_s dosages of ultrasonication (<4400 kJ/kgTS) may enhance sludge dewaterability and above the threshold E_s value dewaterability deteriorates (Feng et al. 2009a). Enhanced dewaterability with low energy dosages (<800 kJ/l) was also reported by Na et al. (2007). The optimal treatment energy was 800 kJ/l above which sludge dewaterability deteriorated. In the current study the threshold value of E_s for the effect of filterability

was not studied, however the dewaterability deteriorated when sludge was ultrasonicated for 5 minutes or more, compared to non-ultrasonicated sludge. Gonze et al. (2003) stated that ultrasound is not applicable upstream of a settling or filtration step in the water treatment process.

Overall, ultrasonic treatment reduces the SVI, but increases the supernatant turbidity. SVI is improved but supernatant turbidity and sludge dewaterability deteriorate. The solubilisation of COD during ultrasonic treatment affects the quality of the supernatant; this is discussed further in Chapter 4.6. The volume of ultrasonicated sludge, the E_s of ultrasonication, and sludge TS affect how settling occurs after ultrasonic pretreatment.

4.3 The effect of ultrasound on particle size distribution

The effect of ultrasound on sludge particle sizes was assessed by measuring the sludge particle size distribution after each ultrasonication in the comprehensive experiments (Figure 4.6). In untreated sludge a major part of the floc particle size was in range between 400 to 700μm. As ultrasonic treatment was carried out and the particle size begun to decrease, the highest concentration of particle size shifted towards 100μm. When ultrasonication time lengthened to 30 minutes, there was a peak at around 100μm and a lower peak at around 500 μm. After 60 minutes of ultrasonication, there were two peaks at 100 μm and around 300-350 μm. This might indicate that the sludge is beginning to re-flocculate or that some of the particles were beginning to be so small that they were not detected by the measurement apparatus. Decreasing particle sizes and re-flocculation phenomenon was also reported in studies by Chu et al. (2001), Gonze et al. (2003), Mao et al. (2004), Bougrier et al. (2005), Dewil et al. (2006), and Feng et al. (2009b).

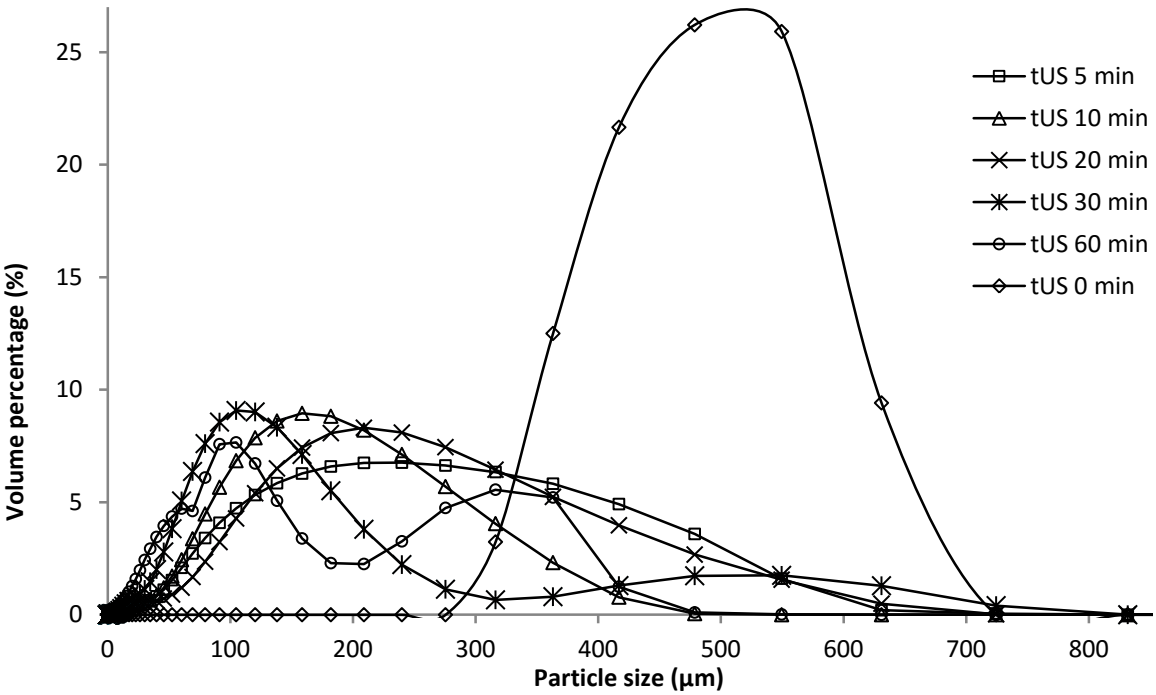

Figure 4.6. *The effect of ultrasound on particle size distribution in the comprehensive experimental set-up.*

In most of other studies, the sludge particle sizes decreased as ultrasonication time was extended or the ultrasonic energy dosage was increased (Chu et al. 2001; Mao et al. 2004; Dewil et al. 2006; Feng et al. 2009b). A threshold value of E_s for sludge disruption was reported by Chu et al. (2001) and Feng et al. (2009b). Chu et al. (2001) observed that 0.11 W/ml ultrasonication had almost no effect on floc size but 0.33 W/ml ultrasonication broke down floc structures. Below the threshold value sludge flocs were disrupted only, and above ultrasound broke up cells, releasing intracellular materials. Feng et al. (2009b) stated that E_s dosages lower than 1000kJ/kg TS did not efficiently disintegrate sludge and that E_s dosages above 5000 kJ/kg TS disintegrated sludge.

The effect of ultrasound on particle size seemed to be the greatest during the first few (1 to 5 minutes) minutes of ultrasonication in this study. Gonze et al. (2003) and Mao et al. (2004) also reported that sludge particle sizes were affected most during first 5 minutes. Chu et al. (2001) found that if the ultrasonic energy dosage is less or equal to 0.11 W/ml, the ultrasonic treatment has almost no effect on the particle size. They also observed that if the ultrasonication was continued for more than 60 minutes, the particle sizes were reduced less than during the previous minutes.

In most of the other studies, the sludge particles were smaller than 100µm in both untreated and treated sludge (Chu et al. 2001; Mao et al. 2004; Dewil et al 2006; Feng et al. 2009b). In this study, particle sizes up to 830 µm were detected. Only Gonze et al. (2003) detected sludge particles greater than this, up to 1000 µm. They suggest that particles with a geometric mean diameter of 0.8±0.2 µm are isolated small micro-

organisms and cellular fragments, micro-flocs and isolated microorganisms have geometric mean diameter of 014±0.5 μm, flocs have geometric mean diameter of 55 to 80 ±10 μm, and macro-flocs and large protozoans have geometric mean diameter of 400±100 μm. Macro-flocs seem to be less resistant to ultrasound than micro-flocs, which are made up of strongly bound bacteria (Gonze et al. 2003).

4.4 The effect of ultrasound on solids and COD

The effects of ultrasound on sludge solids content were measured in both comprehensive and limited experimental set-ups. The sludge samples were measured for TS, VS and SS in comprehensive experiments (Table 4.6, Figure 4.7) and for TS in limited experiments (Table 4.7).

Table 4.6. *The effect of ultrasound on TS, VS, SS in the comprehensive experimental set-up.*

t_{US} (min)	0	5	10	20	30	60
TS (g/l)	8.4	9.8	10.4	10.7	4.5	4.7
VS (g/l)	4.8	6.0	6.4	6.4	2.5	2.9
SS (g/l)	7.5	9.3	9.5	10.4	3.3	4.0
VS/TS (%)	57	62	61	60	54	62
SS/TS (%)	90	96	91	97	72	84

The amount of TS varies during experiments, however the relative share of VS and SS do not change remarkably during ultrasonic treatment. In the current study the VS/TS range was from 54 to 62% and the SS/TS range was from 72 to 97%. In the study by Mao et al. (2004) the VS/TS range stayed at a level of 75-79% indicating that the major part of TS in sludge is organic matter. Both TS and VS remained nearly constant during ultrasonic treatment also in the study by Feng et al. (2009b).

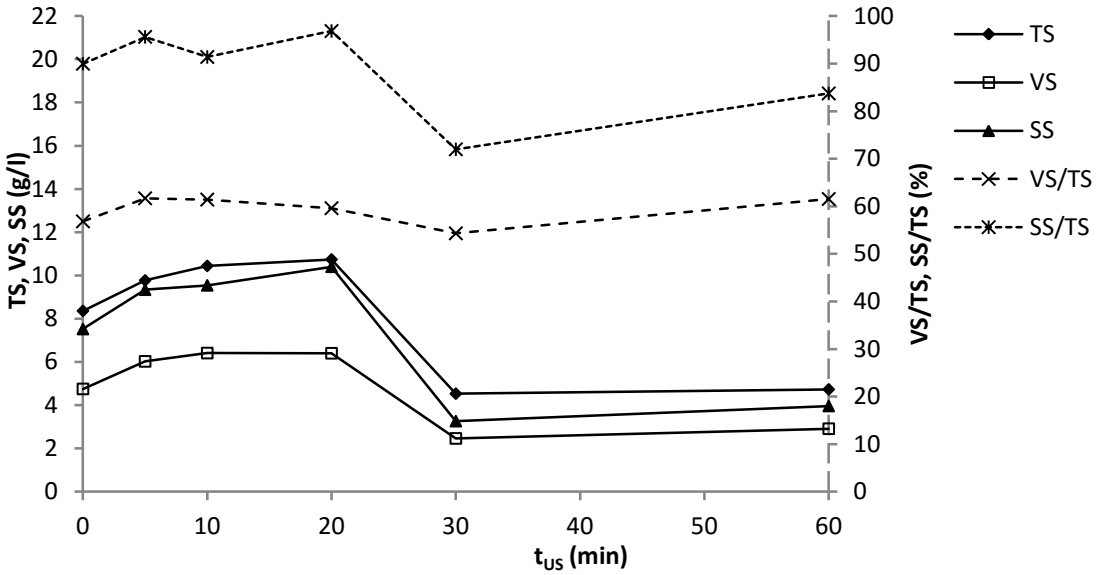

Figure 4.7. *The effect of ultrasound on TS, VS, SS, VS/TS and SS/TS in the comprehensive experiments.*

Bougrier et al. (2005) stated that ultrasound did not induce evaporation phenomenon. However the solubilization of matter was analyzed in their study and they concluded that soluble matter concentration increased whereas particulate matter concentration decreased. Thus both organic solids and mineral solids solubilized during ultrasonication. Solubilization of mineral solids was less than 3% and solubilization of organic solids was 29% (at 15 000 kJ/kg TS) (Bougrier et al. 2005).

Table 4.7. *The effect of ultrasound on TS in the limited experimental set-up.*

t_{US} (min)	0	5	10	20	30	60
TS (g/l)	11.6	11.6	11.3	10.4	11.7	11.7

Evaporation and mineralization phenomenon were not induced in the ultrasonic treatment in the study by El Hadj et al. (2007), in which their TS and SS values remained at almost constant(32.90 and 23.49 g/l). Organic matter (VS) solubilization improved approximately 40% for E_s greater than 11 000 kJ/kg TS (El-Hadj et al 2007). The composition of sludge TS, VS and SS varies between different studies; however, ultrasonic treatment does not affect the solids content in the sludge and the differences which appear in the measurements are due to inaccuracies in the analysis.

The effect of ultrasonic treatment on total and supernatant COD and COD from filtrates was analyzed in comprehensive experimental set-up (Table 4.8, Figure 4.8). Total and supernatant COD were also measured in the limited experimental set-up (Table 4.9, Figure 4.9).

Table 4.8. *The effect of ultrasound on COD, SCOD and COD_f in the comprehensive experimental set-up.*

	t_{US} (min)	0	5	10	20	30	60
TCOD	(mg/l)	5900	8400	8800	9100	4500	4700
TCOD/TS	(mg/g)	710	860	850	840	1000	990
SCOD	(mg/l)	0	1500	3400	4900	4000	5800
SCOD/TS	(mg/g)	0	150	330	460	890	1200
SCOD/TCOD	(%)	0	18	38	54	89	124
COD_f	(mg/l)	49	250	260	560	370	790
COD_f/TS	(mg/g)	5.9	25.6	25.3	52.2	81.5	166.0
COD_f/TCOD	(%)	1	3	3	6	8	17

The SCOD increased as the ultrasonic treatment time became longer. Also the COD_f increased slightly. There is an obvious error in the measurements were the proportional SCOD/TCOD value exceeds 100%, this error is due to inaccuracy in the analysis. However, the results indicate that a significant amount of COD was released into the supernatant during ultrasonic treatment. The matter in solid phase was transferred into aqueous phase increasing the amount of organic matter and EPS in the supernatant.

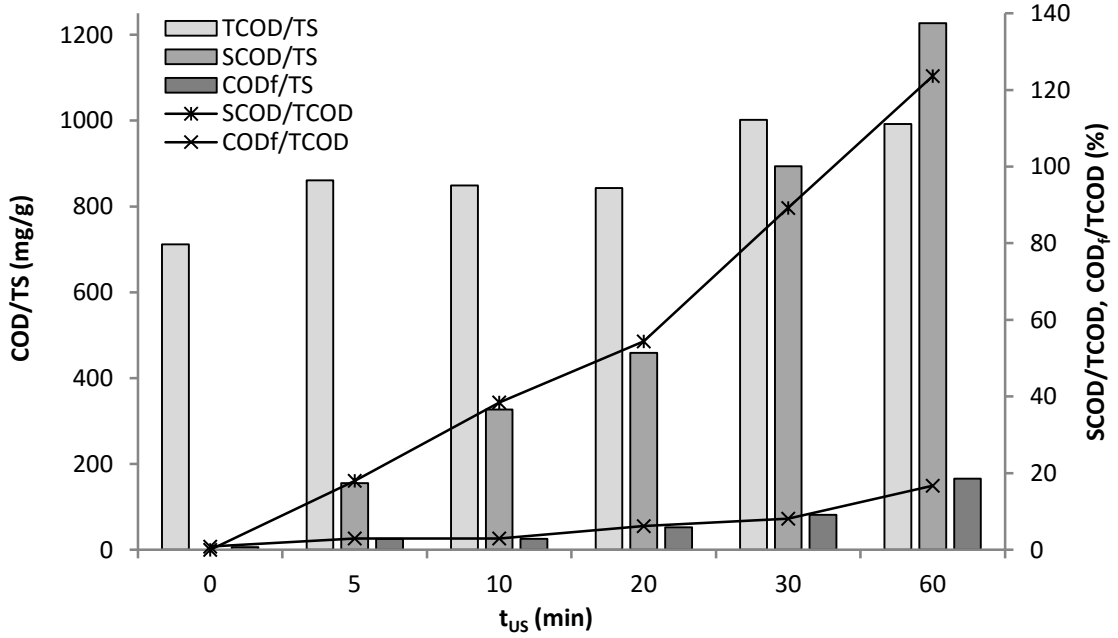

Figure 4.8. *The effect of ultrasound on COD, SCOD and COD_f in the comprehensive experimental set-up.*

Total and supernatant COD in the limited set of experiments confirm the previous conclusion. SCOD in supernatant also increased steadily with increasing E_s in the studies by Gonze et al. (2003), Mao et al. (2004), Gröönroos et al. (2005), Zhang et al. (2007) and Feng et al. (2009b).

Table 4.9. *The effect of ultrasound on COD and SCOD in the limited experimental set-up.*

	t_{US} (min)	0	5	10	20	30	60
TCOD	(mg/l)	4600	4400	4900	4600	4300	4300
TCOD/TS	(mg/g)	390	380	440	440	370	360
SCOD	(mg/l)	290	1000	1500	2400	2700	4100
SCOD/TS	(mg/g)	25	86	130	230	240	350
SCOD/TCOD	(%)	6	23	31	53	66	96

Feng et al. (2009b) reported a positive correlation of over 0.99 between SCOD and the applied energy dosage and Mao et al. (2004) reported a positive correlation of over 0.97 between SCOD and ultrasonication time. In the current study the linear correlation between SCOD/TCOD and ultrasonication time in the comprehensive and limited experiments were 0.94 and 0.95 respectively (Figure 4.10). The results in comprehensive and limited experimental set-ups are parallel and there is only little deviation between the two experimental set-ups.

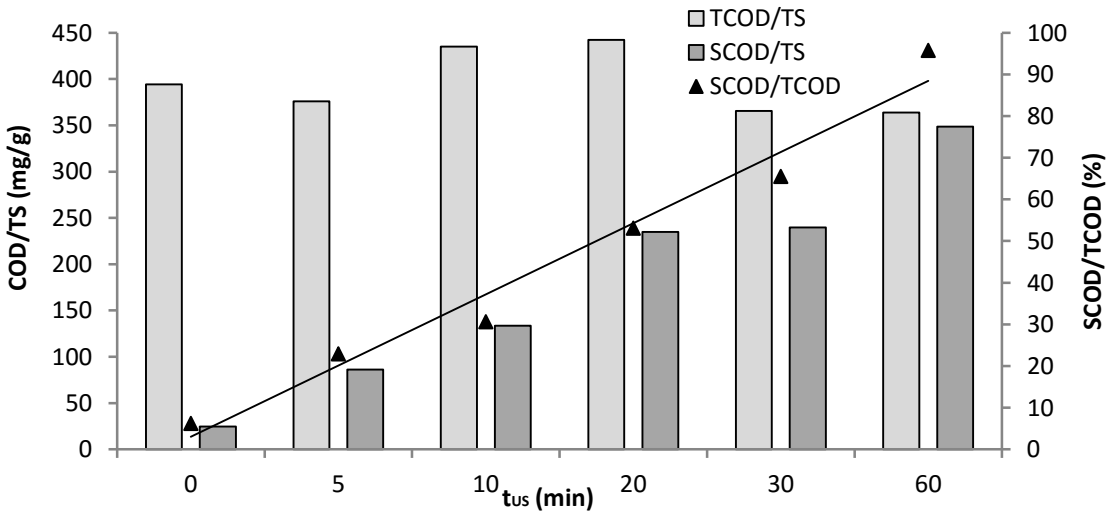

Figure 4.9. *The effect of ultrasound on COD/TS and SCOD/TCOD in the limited experimental set-up.*

A low initial SCOD/TCOD ratio indicates that a large portion of the COD is associated with the solid phase rather than in a soluble form in the aqueous phase (Chu et al. 2001; Mao et al. 2004). The organic material that is solubilized during ultrasonic treatment originates from extracellular organic compounds which are present in the bacterial flocs. Some portion of the SCOD may also originate from intracellular material, which is released during cell lysis. (Gonze et al. 2003; Feng et al. 2009b.)

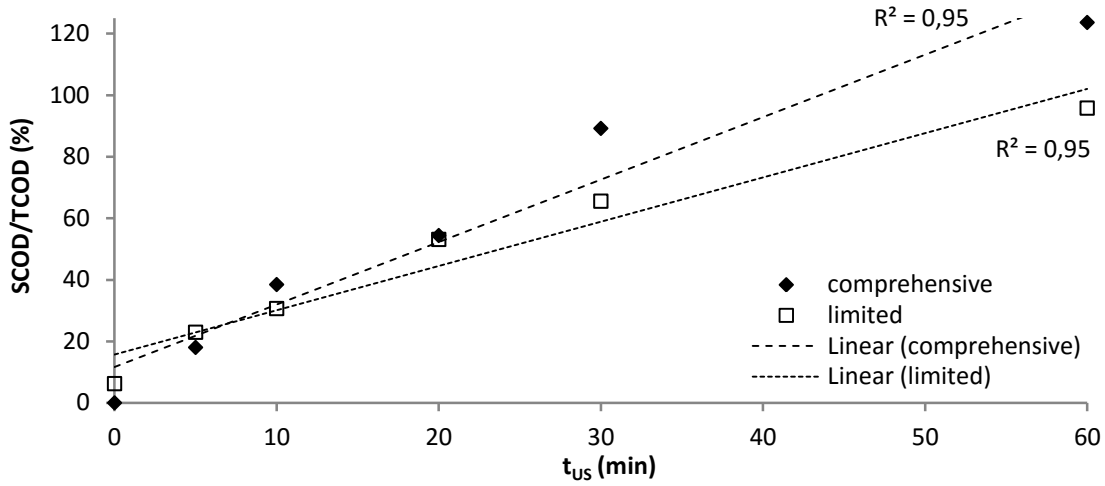

Figure 4.10. *The effect of ultrasound on SCOD/TCOD in comprehensive and limited experimental set-ups.*

If the sludge temperature is allowed to rise during ultrasonication, it gradually also increases the SCOD/TCOD ratio (Figure 4.11). If temperature is controlled and kept steady, the SCOD/TCOD reaches a plateau at approximately 7% and longer ultrasonication does not affect the SCOD/TCOD value, indicating that ultrasound has a limited capability transforming organic material as reported by Chu et al. (2001). When the sludge temperature was allowed to rise up to 60 °C during 120 minutes of ultrasonication, COD was continuously released to the supernatant, but notably, heating alone could not significantly deteriorate the sludge flocs (Chu et al. 2001). At high temperatures both heterotrophic bacteria and total coliform are considerably disinfected, which increases the SCOD values. (Chu et al. 2001; Gröönroos et al. 2005.)

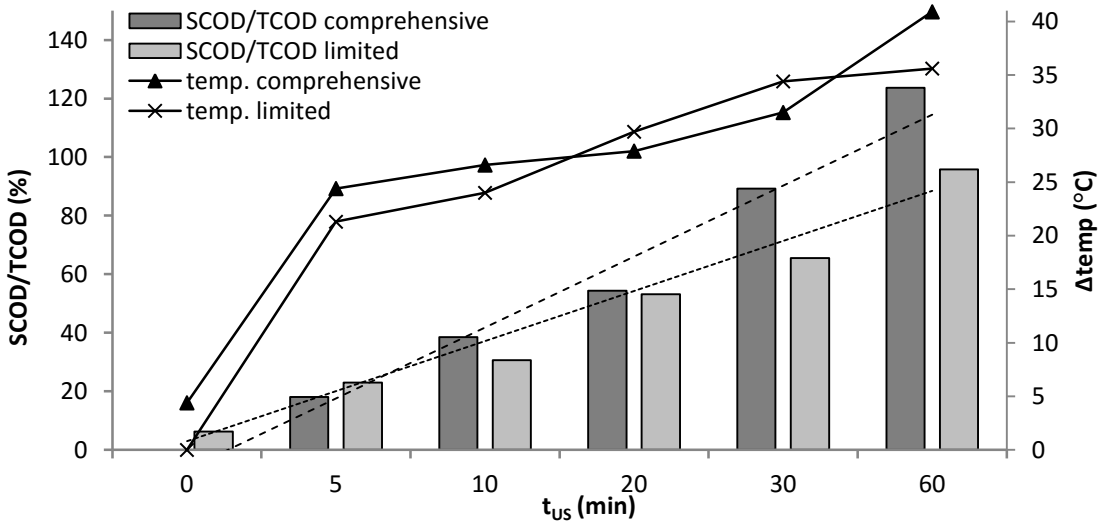

Figure 4.11. *The effect of ultrasound on SCOD/TCOD and Δtemperatures in the comprehensive and limited experimental set-ups.*

Ultrasonic power, ultrasonication time, sludge TS and sludge temperature have a significant positive effect on SCOD. The biggest SCOD increments can be reached with high power, high TS, and long treatment time (Gröönroos et al. 2005). High TS content presents more cavitation sites which produce more hydro-mechanical shear forces. On the other hand beyond a certain TS an optimum level is exceeded, acoustic waves are absorbed and attenuated, and SCOD decrease. Overall, SCOD/TCOD is a good parameter to be used in the evaluation of sludge disintegration. (Mao et al. 2004; Pilli et al. 2011.)

4.5 The effect of ultrasound on biodegradability and carbohydrate content

The effect of ultrasonic treatment on sludge biodegradability was analyzed in the limited experimental set-up (Table 4.10, Figure 4.12). Along with the biodegradability in percentages, the difference in biodegradability between non-ultrasonicated and ultrasonicated sludge samples were also recorded in percentage points (pp) for each ultrasonicated sludge sample.

Table 4.10. *The effect of ultrasound on biodegradability in the limited experimental set-up.*

t_{US} (min)	0	5	10	20	30	60
Biodegradability (%)	15.9	16.0	14.7	6.7	19.3	19.4
ΔBiodegradability (pp)	-	0.1	-1.2	-9.3	3.4	3.5

In the current study, the biodegradability of non-ultrasonicated and ultrasonically treated samples varied irregularly between - 9.3 and 3.5. The BOD of sludge increased with the increasing duration of the ultrasonic treatment which marked that biodegradable organic components were released from sludge particles in the study by Dewil et al. (2006). Mao et al. (2004) concluded that the higher the concentration of SCOD, the better efficiency is expected in the subsequent anaerobic digestion. With a 0.33 W/ml ultrasonication, Chu et al. (2001) reported that about 20% of the TCOD had been transformed into sludge supernatant after 120 minutes of ultrasonication and the BOD/TCOD ratio was increased from 66% to 80% and Pham et al. (2009) reported a 1.4 times higher biogas production with ultrasonicated sludge compared to untreated sludge.

Biomass inactivation occurred after 10 minutes of ultrasonic treatment and ultrasonication time had a notable effect on the biomass inactivation efficiency in the study by Zhang et al. (2007).

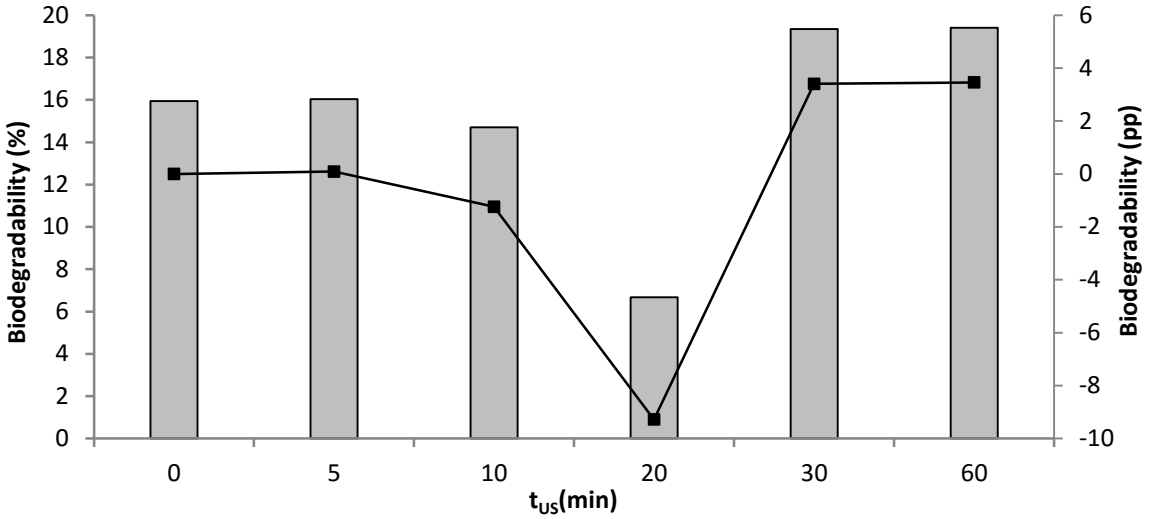

Figure 4.12. *The effect of ultrasound on biodegradability in the limited experimental set-up*

The biodegradability of sludge in this study was stayed at a fairly even level throughout the analysis, except for the obvious decline when sludge was ultrasonicated for 20 minutes. In total, ultrasonic treatment didn't seem to have any effect on the biodegradability of sludge. In reality, the ultrasonic treatment had two opposite effects on sludge which affected the biodegradability. On one hand, ultrasonic treatment enhances sludge solubilization, having a positive effect on the biodegradability and on the other hand, ultrasonic treatment with increasing sludge temperature caused cell lysis and cell inactivation, affecting the biodegradability negatively. Thus the results for biodegradability in this study are inconclusive.

The effect of ultrasonic treatment on the sludge carbohydrate content was measured by using d-Glucose as the standard solution in the comprehensive experimental set-up. First a standard curve was established with d–Glucose (Table 4.11, Figure 4.13). From the standard curve an equation was established with which the carbohydrate content of sludge samples were determined (equation 4.1).

Table 4.11. *The d-Glucose standard curve concentrations and the average absorbances at 490 nm in the comprehensive experimental set-up.*

Target concentration of standard solution (mg/l)	True concentration of standard solution (mg/l)	$\bar{\chi}$ Absorbance at 490 nm
5	4.8	0.0165
10	9.6	0.0130
40	40.8	0.0320
80	78.4	0.0575
120	117.6	0.1150
160	164.0	0.1235
200	199.6	0.1515

The equation with which the carbohydrate contents of the samples can be defined is shown in equation 4.1.

$$y = 0.008 \times x \qquad (4.1)$$

where *y* is the absorption and *x* is the concentration of the sample. The carbohydrate values of ultrasonicated samples can be determined with this equation.

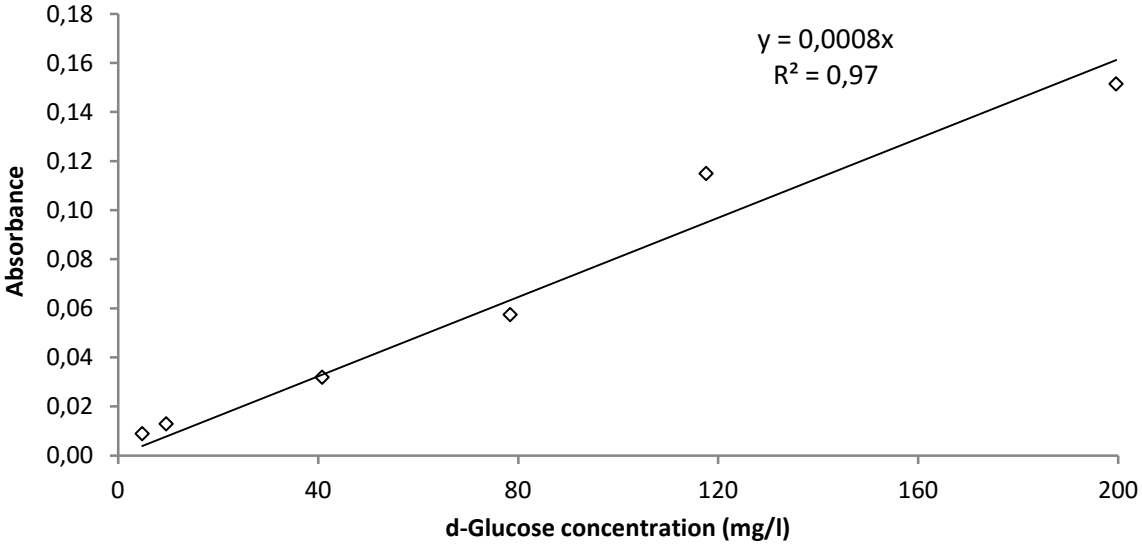

Figure 4.13. *Standard curve of d-Glucose in the comprehensive experimental set-up.*

The concentration of carbohydrates increased almost linearly as the ultrasonication time increased (Table 4.12, Figure 4.14). The trend line linear correlation reached 0.97 suggesting that sludge flocs were disrupted and EPS as well as carbohydrates were released steadily from the sludge flocs during ultrasonic treatment. The release of carbohydrates possibly enhance the subsequent biodegradability.

Table 4.12. *The effect of ultrasound on carbohydrate concentrations in the comprehensive experimental set-up.*

t_{US} (min)	0	5	10	20	30	60
Carbohydrate concentration (mg/l)	20.0	101.3	129.4	223.8	237.5	308.1

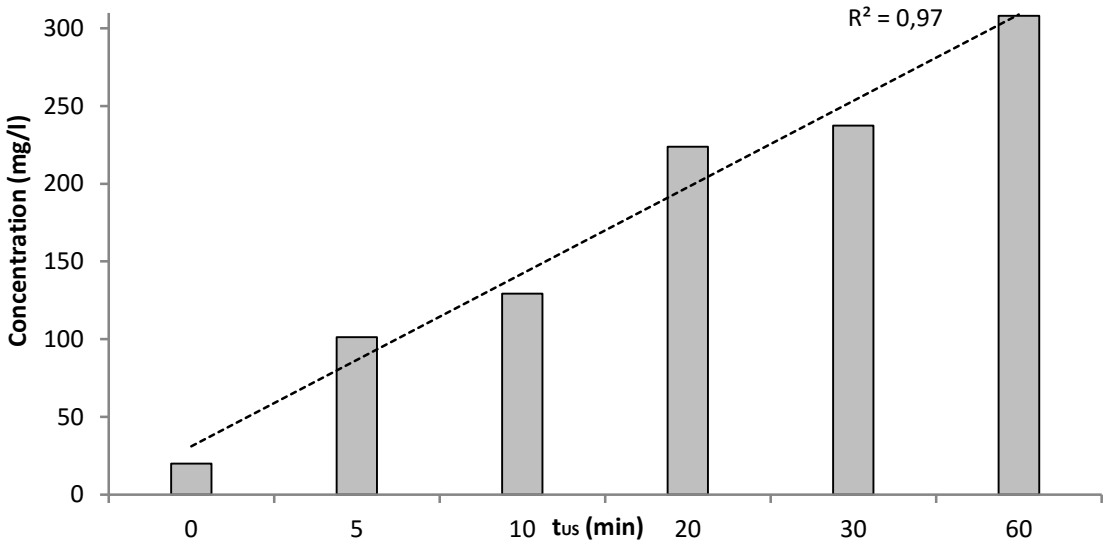

Figure 4.14. *The effect of ultrasound on the concentration of carbohydrates in the comprehensive experimental set-up.*

5 Conclusions

This study characterized waste activated sludge before and after ultrasonic pretreatment. Characterization methods included temperature, particle size distribution, TTF, settling, SVI, supernatant turbidity, solids content, COD, biodegradability and carbohydrate content.

Ultrasonic treatment increased sludge temperature. The temperature increased most during first 5 minutes of ultrasonication after which the temperature continued to rise less, but somewhat linearly. Both ultrasonication and rising temperature increased linearly the SCOD and carbohydrate content in sludge supernatant, and COD_f in sludge filtrate was also increased but at a lower rate than SCOD. SCOD and COD_f indicated that organic matter was released from sludge particles and EPS and became available for subsequent aerobic or anaerobic digestion. Rising temperature and ultrasonic treatment also disinfected sludge and caused microbial inactivation affecting sludge biodegradability by decreasing it when biodegradability was carried out with utrasonicated samples and without adding fresh and active microbial inoculums to the samples before biodegradability analysis. The biodegradability of sludge samples remained at the same level despite the length of the ultrasonic treatment, even though it was expected that ultrasonic treatment would significantly increase the biodegradability. The results gained in the biodegradability analyses therefore were not conclusive.

The effect of heating should be taken into account in ultrasonic experiments and a sufficient cooling system should be added especially if heating is an unwanted effect. Relying on research results in other studies and provided that increased biodegradability is desired, the determination of SCOD is a good and easy method for the evaluation of biodegradability. The analysis of carbohydrate content is also a good indicator however, the experimental methods are much more complex.

Ultrasonic treatment tore down EPS, releasing water and reducing particle sizes which increased the cohesion and density of particles accelerating the settling of sludge. Settling was faster especially with longer (> 20 min) ultrasonic treatment times and the SVIs decreased almost linearly throughout the ultrasonic experiments. On the other hand, the release of small particles and colloids that had a density close to that of water did not settle and caused clouding of the supernatant. Particle size distribution shifted towards smaller particle sizes as ultrasonication treatment time became longer. When ultrasonic treatment times were long (> 20 min), re-flocculation phenomenon was also observed.

Ultrasonication deteriorated values in TTF, which increased substantially after only 5 minutes of ultrasonic treatment, thus ultrasonic treatment for enhancing sludge dewaterability is not advised. Ultrasonic treatment had no effect on the relative shares of TS, VS and SS which remained at a fairly constant level throughout the ultrasonic experiments.

Results in this study are directional but not explicit and definite. Most of the results in the current study are consistent with the results of other studies which were reviewed in this study. Differences in results are due to different ultrasonication operation conditions and ultrasonic experiments outcome depend on ultrasonication parameters including the ultrasonic input power and duration, along with sludge TS treatment volumes. Reactor type and design also have an impact on how sludge is influenced. Depending on the desired effects, ultrasonic treatments need to be designed case specifically. At best the ultrasonication specific energy should be determined ad hoc since the composition of sludge in waste water treatment varies according to treatment conditions. For these reasons ultrasonic pretreatment should be studied more in full scale applications to establish if operational conditions of the ultrasonic treatment can be optimized well enough to achieve enhanced sludge characteristics with a cost-effective treatment.

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